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PROCEEDINGS OF THE THIRTY-FIRST ANNUAL CONVENTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1914.

THIRD DAY.

WEDNESDAY—MORNING SESSION.

No report was made by the referee on the general subject of separation of nitrogenous substances.

REPORT ON SEPARATION OF NITROGENOUS SUBSTANCES (MILK AND CHEESE).

By Leroy S. Palmer (Agricultural Experiment Station, Columbia, Mo.), *Associate Referee*.[1]

The referee on separation of nitrogenous substances (milk and cheese) was appointed in March to fill the vacancy due to the resignation of O. B. Winter, and there was not time for definite coöperative work before the close of this fiscal year.

No recommendations have been made since 1911 and, as far as could be learned by the associate referee, no work has been done along the line of the separation of the amino acids in cheese recommended at that time. It was decided, therefore, with the consent of the referee on the separation of nitrogenous substances, to lay plans for taking up work of a more general nature, where it was felt there was some need.

The associate referee has been impressed for some time with the need of a careful study of the determination of the so-called albumin of milk, leading if possible to the adoption of an accurate, reliable method, and, as associate referee, at once saw the opportunity of obtaining the coöperation of the association in this subject as no official method has ever been adopted. L. L. Van Slyke, who devised the present provisional method, as referee in 1908, recommended for study during the following year the selection of an official method for the determination of milk albumin. The recommendation either was not approved or did not come up in time for approval. Further evidence of need along this line is shown by the frequently recurring articles in the chemical journals, of improved methods for milk albumin. One which appeared during the past year[1] pointed out the varying results obtained by the provisional method of this association. To obtain further evidence a letter was sent to the chemists who had indicated their willingness to coöperate in the milk and cheese protein work containing the following inquiries:

---

[1] Presented by P. F. Trowbridge.

1. Do you use the provisional method of the Association of Official Agricultural Chemists for milk albumin?
2. If so, do you find it entirely satisfactory?
3. If not, what modification do you use and what is its basis?

Three of the four coöperators replied as follows:

R. E. Stallings: We use the provisional method in this laboratory.

C. O. Swanson: We do not find the provisional method for milk albumin satisfactory because it is almost impossible to get constant results. The amount of albumin obtained depends to such a large extent upon conditions of temperature, time of heating, and the personal equation that it is very difficult to get comparative results. We have not worked out any modification of the method.

A. W. Bosworth: We use the provisional method for the determination of albumin in milk. The method originated in this laboratory and, although it is not entirely satisfactory, it seems to be the best one available.

It is now purposed to make some recommendations to the association in regard to milk albumin, but before doing so attention is called to a point in the nomenclature of the milk proteins, especially in regard to the so-called albumin. To any one familiar with the composition of milk it is obviously not strictly correct to call the heat-coagulable protein of milk, albumin, when there is recognized in milk the presence of a heat-coagulable globulin which is precipitated with the albumin in the present provisional method. It has always seemed that the more correct nomenclature of the milk proteins, as determined by the present methods, should contain the terms casein and heat-coagulable proteins (albumin and globulin) in place of the present terms casein and albumin.

If the association approves the recommendation to make studies leading to the selection of an official method for milk albumin, the question arises as to the definition of the term albumin, whether strictly as lact-albumin, or in the sense in which it is used at present, that is, heat-coagulable protein. The associate referee is inclined to think that a separate determination of albumin and globulin would not be wise, but that better success lies in the improvement of the present provisional method for the so-called albumin. Should the association agree, it is suggested that the matter of changing the nomenclature be taken up with the view of indicating the correct character of the protein obtained on coagulation with heat.

---

[1] *J. Ind. Eng. Chem.*, 1914, **6**: 573.

Another matter concerning the milk proteins that is believed would be profitable for the association to consider is the selection of official methods for the determination of the protein substances in milk other than casein and the heat-coagulable albumin and globulin. Reference has not been made to all the association reports, but in 1901 Mr. Le Clerc, referee on dairy products, called attention to the fact that the protein substances in milk, not precipitated by acetic acid or heat, amount in many cases to from 10 to 12 per cent of the total protein. Mr. Le Clerc urged at that time that some attention be given to the determination of these bodies. The determination of these substances which we recognize as caseoses, albumoses, globuloses, peptones and other protein-like substances and nitrogenous extractives, is important not only because of their presence in fresh milk, but especially because of their increase in milk after it is drawn, due to the action of proteolytic enzymes. A study[1] of their relative proportion in milk of various states of freshness and under other conditions leads to important results from the standpoint of the nutritive value of the milk. While it would probably not be feasible for the food chemist to go into the separation of the individual members of this group of substances, it would be a very simple matter to separate them into two groups: namely, (1) the proteoses and peptones; and (2) the remaining nitrogenous substances, which might be called residual proteins. Such a separation is readily made by tannic acid. A number of analyses made in this manner have shown that tannic acid divides the non-casein non-heat-coagulable proteins into approximately equal parts, both of which are usually greatly increased in old milk.

In conclusion attention is called to the fact that studies in the preservation of milk for chemical analysis, which have long been of interest to this association, are often useless inasmuch as the methods for the analysis of the proteins remain unsatisfactory. It is urged, therefore, that the association take up these matters for study. The following recommendations are accordingly offered for approval.

RECOMMENDATIONS.

It is recommended—

(1) That the nomenclature of the so-called albumin of milk be changed to heat-coagulable proteins (albumin and globulin) in order that the true character of the protein thus obtained be shown.

(2) That studies be made leading to the adoption of an official method for the determination of the heat-coagulable proteins (albumin and globulin) of milk.

(3) That studies be made leading to the adoption of methods for the determination of the proteoses, peptones, and residual nitrogenous substances in milk.

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[1] *J. Biol. Chem.*, 1913, **16**: 331.

THE SO-CALLED NEUTRALIZATION PRECIPITATE OF COWS' MILK.

By Leroy S. Palmer (Agricultural Experiment Station, Columbia, Mo.).

Van Slyke and Hart,[1] in 1903, called the attention of this association to the fact that the filtrate from the acetic acid precipitation of the casein of cows' milk (by the official association method) gives more or less of a precipitate on neutralization. They stated that this precipitate usually amounted to 0.1 per cent of casein. The explanation offered for this phenomenon was as follows: When cows' milk is treated with 1.5 per cent acetic acid, as in the official method, the precipitate formed is wholly casein-diacetate. A small portion of this is dissolved by the excess acetic acid used and reappears when its solution, the filtrate from the casein precipitation, is neutralized. Whether this neutralization precipitate is in the form of casein or its acetic acid compound was not stated.

Later work by Van Slyke and Van Slyke[2] on the action of dilute acids upon casein, would indicate that this explanation requires some modification. These investigators definitely showed that casein does form acetic acid, as well as other acid compounds, which are somewhat soluble in an excess of the acid used. That these acid compounds are of a definite character, however, was not borne out by the investigation. They appeared to be adsorption compounds rather than chemical combinations. It was also shown that the acid-casein compounds, when in solution, give up their acid on neutralization. It would appear from this that the neutralization precipitate, if it arises from an acid-soluble casein-acetate, should be free from acetic acid.

Interest in the so-called neutralization precipitate grew out of its invariable appearance, in greater or less amount, in some studies of new methods for the determination of the heat-coagulable proteins of cows' milk, in all of which the casein was first removed by acetic acid. It seemed possible that the protein character of the neutralization precipitate, as indicated by the previously mentioned work, might have a bearing on the determination of the casein or the heat-coagulable proteins.

Van Slyke and Van Slyke showed that the precipitate, obtained on careful neutralization of solutions of casein-acid compounds, had all the properties of casein, being readily soluble in an excess of alkali, and reappearing again on neutralization. They also found that the precipitation of the casein on addition of alkali was practically complete before the neutral point was reached, but could be prevented entirely if the alkali was added rapidly.

[1] Bur. Chem. Bull. 81, p. 91.
[2] *Am. Chem. J.*, 1907, **38**: 383.

A brief study of the properties of the neutralization precipitate obtained on addition of sodium hydroxid to the casein filtrate showed that it also possessed the property of being precipitated before neutrality was reached, and of dissolving again on addition of acid. It was not found possible, however, to bring about a solution of the precipitate in sodium hydroxid. This insolubility in sodium hydroxid did not appear to be affected in any way either (a) by the manner of adding the alkali, whether rapidly or drop by drop, or (b) by the amount of sodium hydroxid added, or (c) by the temperature of the solution. In fact practically no solution occurred even on long boiling in sodium hydroxid solution. This treatment, however, resulted in rendering some of the precipitate insoluble in acetic acid.

The question at once arose, is the so-called neutralization precipitate casein?

As a preliminary investigation, casein was prepared from milk by the addition of acetic acid, according to the method used by Van Slyke and Van Slyke. Solutions of this casein and also of Merck's c. p. casein (according to Hammarsten) were made in sodium hydroxid and in glacial acetic acid. These solutions were then neutralized with acetic acid and sodium hydroxid, respectively, and the casein precipitated from the neutral solutions by acetic acid under conditions as nearly identical as possible with those which apparently cause the formation of the neutralization precipitate in the case of milk. The filtrates were then examined for neutralization precipitate. None was found in any case.

This result also throws great doubt upon the supposition that the neutralization precipitate is casein. On the other hand, the properties of the neutralization precipitate already described were so suggestive of tricalcium phosphate that a calcium and phosphorus analysis seemed warranted. For this analysis the neutralization precipitate was isolated from one liter of skim milk as follows:

One liter of fresh skim milk was diluted with one-half liter of water, and 150 cc. of 10 per cent acetic acid added, the diluted milk being at a temperature of 40°C. After cooling, the casein was filtered off. Several filtrations were required to get a clear filtrate. Sodium hydroxid solution (10 to 20 per cent) was now added to the filtrate to a faint alkaline reaction (to phenolphthalein). A heavy flocculent precipitate appeared. It was filtered off, washed somewhat with water, and then suspended in water to which 40 cc. of 10 per cent acetic acid had been added. All but traces of the precipitate dissolved readily in this solution. The acid solution was now placed on the steam-bath for an hour and then boiled for several minutes. The coagulated proteins thus formed were removed by filtration. The filtrate was treated with 10 to 20 per cent sodium hydroxid solution to faint alkalinity. A heavy flocculent precipitate came down, most of it before the neutral point was reached. The neutralization precipitate was filtered off, washed thoroughly on the paper, and then suspended in water and dissolved by the addition of dilute acetic acid. Sodium hydroxid was added to this solution, bringing about reprecipitation. This precipitate was washed many times by decantation. It was finally dried on a porous plate in the electric oven at 105°C., yielding a fine, pure white powder, which was dried to constant weight at 105°C. before analysis. About 1.8 grams of dry material were obtained, no allowance being made for losses in isolation.

The results of the analysis of the neutralization precipitate are here given. On ignition at red heat it gave off water and blackened. After burning off the carbonaceous matter the ash was analyzed for calcium and phosphorus.

	Per cent
Total loss on ignition	16.92
Water lost on ignition	6.95
Nitrogen in dry substance	0.46
Calcium in ash	36.16
Phosphorus in ash	21.12

The results were found rather difficult to interpret because of the large loss on ignition. A calculation based on the results obtained, however, shows that a mixture of 72.5 per cent of $Ca_3(PO_4)_2$, 24.5 per cent of $CaHPO_4$, 2 per cent of H_2O and 2.8 per cent of protein (casein or albumin) would give results conforming very closely to those obtained. The relation between the theoretical analysis of such a mixture and the results found is indicated in the following table:

	Theoretical mixture per cent	*Found for neutralization precipitate per cent*
Calcium	33.55	32.60
Phosphorus	19.55	19.10
Water lost on ignition	6.57	6.57
Nitrogen	0.46	0.46

If the protein in the precipitate be considered as casein, the relation between the theoretical results obtained from the ash of such a mixture and the results obtained for the neutralization precipitate would be those given in the following statement:

ASH ANALYSIS.

	Theoretical mixture per cent	*Neutralization precipitate per cent*
Calcium	36.18	38.45
Phosphorus	21.13	22.58

The greater difference in this case is due to the unexplained loss on ignition.

These results make untenable the previously advanced explanation of the origin and character of the so-called neutralization precipitate of cows' milk. Instead of being a portion of the casein which has been dissolved by the excess of acid used, it appears to be almost entirely a mixture of di- and tricalcium phosphates. Whether the protein obtained in the analyses is a contamination or an essential part of the precipitate is not apparent.

It may be stated, however, that the method of isolation would seem to preclude a protein contamination, which is also borne out by the ready solubility of the isolated precipitate in acetic acid.

The source of the neutralization precipitate is a question of considerable interest, especially since some evidence can be presented in regard to it. Lindet[1] has recently shown that casein, which has been precipitated from cows' milk by rennet, will yield CaO and P_2O_5 on treatment with dilute acetic acid, equivalent to 3.45 per cent $Ca_3(PO_4)_2$ and an additional 1.84 per cent CaO. Since this result is obtained merely by washing with dilute acid, it would seem that the explanation of the origin of the calcium phosphate of the neutralization precipitate is very simple. It is possible, of course, that some of the calcium phosphate, especially the dicalcium phosphate, may result from preëxisting phosphates in the milk serum. In view of Lindet's results and the fact that it was not found possible to bring about a further liberation of calcium phosphate (neutralization precipitate) from casein which had been thrown down by acetic acid, it would seem to be a very easy matter to demonstrate whether the source of the neutralization precipitate is the caseinogen of the milk or preëxisting phosphates of the serum, or whether it arises from both of these sources. According to Lindet's results, casein precipitated by rennet should yield a neutralization precipitate after treatment with acetic acid; and if this is the only source of the neutralization precipitate, the filtrate from the rennet coagulation should yield no neutralization precipitate. In the acetic acid coagulation of casein, the filtrate from the coagulation of the albumin and globulin by heat will usually show the neutralization precipitate as readily as the filtrate from the casein coagulation.

No difficulty was experienced in showing the absence of neutralization precipitate in the filtrate from the rennet coagulation of casein, either before or after coagulation of the albumin and globulin with heat. On the other hand, neither was it found possible to show that rennet-coagulated casein will yield a neutralization precipitate after treatment with acetic acid. In this study practically the same procedure was followed as in the previous study of the acid-coagulated casein, with the exception that ammonium hydroxid[2] was used for the alkaline solvent in place of sodium hydroxid. Calcium phosphate being somewhat soluble in ammonium acetate solution, it was necessary in this case to remove the ammonia by distillation in the presence of fixed alkali, before testing for the neutralization precipitate.

The explanation of this result is not apparent. Possibly the conditions were not right, or the calcium phosphates obtained in the usual neutralization precipitate are the result of a chemical reaction and not of a mere washing with dilute acetic acid. Even then, it is not apparent why a repetition of Lindet's work did not yield a precipitate of calcium phosphate on neutralization.

---

[1] Proc. 8th Intern. Cong. Appl. Chem., 1912, **19**: 199.

[2] Lindet states that rennet-coagulated casein dissolves in ammonium hydroxid without liberation of calcium phosphate.

CONCLUSIONS.

(1) The so-called neutralization precipitate obtained from the filtrate of the acetic acid coagulation of cows' milk appears to be largely a mixture of di- and tricalcium phosphates. The precipitate isolated in this study was mixed with a small proportion of protein, probably casein or albumin, either as a contamination or as an essential part of the precipitate.

(2) The origin of the calcium phosphates of the neutralization precipitate would presumably be largely the caseinogen of the milk, but attempts to show this fact, using casein coagulated by rennet, have so far been unsuccessful.

(3) The very small amount of protein contained in the neutralization precipitate precludes its being an important factor in the determination of casein or the heat-coagulable proteins.

REPORT ON DAIRY PRODUCTS.

By **Lewis I. Nurenberg** (State Board of Health, Boston, Mass.), *Referee.*[1]

The following recommendations were made for collaborative work:

(1) That the copper sulphate method of preparing milk serum be studied with a view of its adoption as an optional provisional method.

(2) That the Harding-Parkin method for fat determination be studied in comparison with the present official and provisional methods.

(3) That in U. S. Bur. Chem. Bull. 107, Rev., p. 122, after the paragraph on Cream, the following paragraph be inserted under the heading "Condensed Milk (Unsweetened)": "Dilute 40 grams of the homogeneous material with 60 grams of distilled water, and proceed as directed under 'Milk', and correct the results for dilution"; and that the word "Sweetened" be inserted before the word "Condensed" in the subsequent heading.

(4) That subjects now under consideration be given further study.

No work on the copper sulphate method has been reported to the association since 1909. In the study of the copper sulphate method it was the referee's idea to collect and compile figures from all available sources. Twenty-two letters were sent out to the various food and dairy departments, asking for figures on known purity milk by the copper refraction method, together with figures on solids, fat, etc., on the same milk, and if the milk was from individual cows, the name of breed and amount of milk given at the time of milking; if from a herd, the number and breed of cows and the amount of milk produced at the time of milking. Figures were also requested on the comparison of the copper and acetic methods for the preparation of milk serum, comparison of the copper method on night's and morning's milk, and on the different stages of lactation. Eleven replies were received, two of which contained some of the desired figures. One reply was from H. Louis Jackson of the Idaho Board of Health, and the other was from E. M. Bailey of the Connecticut Agricultural Experiment Station. The referee has obtained figures on 834 samples of known purity milk, examined during the past eight years in the laboratory of food and drug inspection of the Massachusetts Board of Health, and, unless otherwise stated, all analyses are from that laboratory.

---

[1] Presented by H. C. Lythgoe.

In 698 cases where the samples came from individual cows the breed was known and is distributed as follows: Jersey 47; Guernsey 38; Grade Jersey 45; Grade Guernsey 26; Grade Durham 27; Grade Ayrshire 54; Swiss 12; Grade Holstein 198; other grades 13; Ayrshire 48; Dutch Belt 51; and Holstein 139. A little more than one-half of the total number, therefore, represent low grade milk. The samples were collected during different seasons of the year and during varying periods of lactation, so that it is proper to consider them as fairly representative of all influencing conditions.

PREPARATION OF THE COPPER SERUM.[1]

Dissolve 72.5 grams of copper sulphate in water and make up to 1 liter. Adjust this solution to read 36 at 20°C. on the scale of the Zeiss immersion refractometer. Add 4 volumes of milk to 1 of the copper sulphate solution, shake well and filter. Determine the index of refraction of the filtrate at 20°C. A reading below 36 is indicative of added water.

Table 1 presents the refractions of the copper sulphate serums on 834 samples of known purity milk. The variation in readings extends from 36–40.4. The largest number of samples, 16.7 per cent, refracts between 37.8 and 38.0, only 3.1 per cent from 36–36.2 and practically none above 40. The range of readings is, therefore, small, much less than that of the acetic serum which extends from 39–46.

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[1] Mass. State Board of Health Rept., 1908, p. 594; U. S. Bur. Chem. Bull. 132, p. 122.

TABLE 1.

Refractions of copper sulphate serum on 834[1] samples of milk of known purity.

READING	NUMBER OF SAMPLES	READING	NUMBER OF SAMPLES	READING	NUMBER OF SAMPLES
36.0	14	37.5	27	39.0	28
36.1	9	37.6	36	39.1	4
36.2	3	37.7	38	39.2	8
36.3	8	37.8	28	39.3	8
36.4	10	37.9	39	39.4	5
36.5	15	38.0	72	39.5	5
36.6	19	38.1	32	39.6	1
36.7	10	38.2	41	39.7	7
36.8	22	38.3	40	39.8	1
36.9	13	38.4	33	39.9	..
37.0	24	38.5	33	40.0	1
37.1	18	38.6	23	40.1	2
37.2	28	38.7	24	40.2	..
37.3	28	38.8	22	40.3	1
37.4	33	38.9	20	40.4	1

[1] This figure includes 53 samples from H. Louis Jackson, State board of health, Boise, Idaho, 53 from W. G. Tice, State board of health, Trenton, N. J. (U. S. Bur. Chem. Bull., 132, p. 130) and 14 from E. M. Bailey, agricultural experiment station, New Haven, Conn.

TABLE 2.

Composition of milk of known purity.

(H. Louis Jackson, Idaho.)

SPECIFIC GRAVITY AT 15.5°C. (WESTPHAL)	FAT BY BABCOCK METHOD (TESTED BOTTLES)	TOTAL SOLIDS[1]	SOLIDS NOT FAT	CORRECTED COPPER SERUM READING AT 20°C.
	per cent	*per cent*	*per cent*	
1.0307	4.1	12.61	8.51	37.2
1.0313	4.5	13.24	8.74	38.1
1.0314	4.2	12.90	8.70	38.0
1.0314	6.5	15.66	9.16	37.9
1.0296	5.0	13.41	8.41	38.5
1.0327	7.9	17.67	9.77	38.8
1.0293	4.5	12.74	8.24	37.4
1.0318	5.5	14.57	9.07	38.7
1.0324	5.5	14.72	9.22	38.4
1.0297	3.6	11.76	8.16	37.2
1.0316	4.5	13.31	8.81	37.9
1.0312	5.1	13.94	8.84	37.6
1.0324	4.1	13.03	8.93	38.2

[1] Leach. Food Inspection and Analysis. 1913, 3rd ed., p. 154.

TABLE 3.
Composition of milk of known purity.
(H. Louis Jackson, Idaho.)

FAT BY BABCOCK METHOD (TESTED BOTTLES)	TOTAL SOLIDS (5 GRAMS FOR 3 HOURS ON THE WATER-BATH)	SOLIDS NOT FAT	CORRECTED COPPER SERUM READING AT 20°C.
per cent	*per cent*	*per cent*	
3.80	12.57	8.77	38.9
3.55	11.39	7.84	38.7
3.35	12.41	9.06	38.5
3.25	11.77	8.52	38.4
3.40	11.36	7.96	36.3
3.30	11.73	8.43	38.5
3.60	11.58	7 98	....
4.60	13.52	8.92	38.0
4.40	13.31	8.91	38.0
3.40	11.52	8.12	....
3.80	12.76	8.96	38.0
3.95	12.60	8.65	38.2
3.70	12.80	9.10	38.3
6.40	15.25	8.85	39.5
3.50	12.29	8.79	38.3
3.50	12.23	8.73	38.0
3.30	11.87	8.57	37.5
3.80	12.61	8.81	37.8
0.88[1]	9.34	8.46	36.5
3.60[2]	12.71	9.11	38.5
10.00[3]	18.53	8.53	38.3
3.85[4]	12.38	8.53	37.8
3.35[5]	11.60	8.25	37.7
3.95[6]	12.90	8.95	38.0
4.00[7]	12.86	8.86	37.7

[1] Fore milk. Just after milking began.
[2] Drawn at the middle of the milking.
[3] Strippings.
[4] From first 10 gallon can filled.
[5] From next 5 gallon can filled.
[6] From next 5 gallon can filled.
[7] From next 5 gallon can three-fourths filled.

TABLE 4.
Composition of milk of practically known purity.
(H. Louis Jackson, Idaho.)

SPECIFIC GRAVITY AT $\frac{15° C.}{15°}$ (WESTPHAL)	FAT BY BABCOCK METHOD (TESTED BOTTLES)	TOTAL SOLIDS BY CALCULATION	SOLIDS NOT FAT	CORRECTED COPPER SERUM READING AT 20°C.	REMARKS
	per cent	*per cent*	*per cent*		
1.0335	3.9	12.06	8.16	38.4	
......	4.6	.....	....	38.6	Night's milk.
......	5.6	.....	....	38.75	Morning's milk.
......	4.28	.....	....	38.2	Morning's milk.
......	6.4	.....	....	38.9	Morning's milk.
......	3.3	.....	....	38.96	Morning's milk.
......	4.6	.....	....	38.0	Morning's milk.
......	5.1	.....	....	40.3	Morning's milk.
......	4.8	.....	....	37.0	Morning's milk.
......	6.0	.....	....	40.1	
......	4.25	.....	....	38.6	Composite of 4 cows.[1]

[1] No. 1, ⅞ Black Jersey ⅛ Holstein, 1 gallon of milk; No. 2, full blood Black Jersey, 2 gallons of milk; No. 3, ¼ Short Horn ¾ Durham, ½ gallon of milk; No. 4, ½ Black Jersey ½ Short Horn, 3 gallons of milk.

Milk from pure Guernsey from registered stock (age 10–11 years).

PART OF UDDER	FAT BY BABCOCK METHOD (TESTED BOTTLES)	TOTAL SOLIDS (5 GRAMS FOR 3 HOURS ON WATER-BATH)	SOLIDS NOT FAT	CORRECTED COPPER SERUM READING AT 20°C.
	per cent	*per cent*	*per cent*	
Left hind teat................	6.13	14.88	8.75	37.62
Left front teat................	6.3	14.96	8.66	37.79
Right front teat................	4.15	13.45	9.30	38.36
Right hind teat................	8.23	17.24	9.01	37.95

In the preceding tables it will be noticed that the lowest copper refraction from the milk of individual cows is 36.3, the highest 40.1, while the herd milk refracts at practically 38.0.

E. M. Bailey of New Haven sent in copper refraction figures on the milk of 14 herds of Holstein and Grade Guernsey cows. The range of the copper readings extended from 36.8–39.4.

The copper serum method besides having a narrow range of readings has several other advantages; it is simple, the serum is very quickly prepared without the use of heat, no loss by evaporation and the determination may be made on a small quantity of milk, sufficient serum being obtained with 17.6 cc. of milk and 4.4 cc. of copper sulphate solution.

PREPARATION OF ACETIC SERUM.[1]

The refractions of the acetic serums on 554 samples of known purity milk are shown in Table 5. From this table it will be noted that the greatest percentage of samples refracts between 43 and 43.5, practically none below 40, and from 4–5 per cent about 45.

During the past five years in the laboratory of the Massachusetts Board of Health the refractive index has been determined on the sour serum of milk.

PREPARATION OF SOUR SERUM.[2]

Allow the milk to sour spontaneously, filter and determine the index of refraction of the clear serum at 20°C. by means of the Zeiss immersion refractometer. Refractive indices have been made on 515 samples of known purity milk and are given in Table 6. About 50 per cent of these samples refract between 41.0 and 43.0.

The number of refractions between 40.0 and 41.0 and between 43.0 and 44.0 is nearly the same, that is, 15 per cent. Five per cent of the samples are between 38.0 and 39.0 and 1 per cent is above 45.0. This method has been found especially useful in cases where a sample of milk presents the least sign of souring.

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[1] Provisional method. U. S. Bur. Chem. Bull. 107, Rev., p. 120.

[2] *Z. offent. Chem.*, 1903, 9: 173.

TABLE 5.

Refraction of acetic acid serum on 557[1] samples of milk of known purity.

READING	NUMBER OF SAMPLES	READING	NUMBER OF SAMPLES	READING	NUMBER OF SAMPLES	READING	NUMBER OF SAMPLES
39.0	1	41.0	13	43.0	42	45.0	4
39.1	..	41.1	4	43.1	14	45.1	2
39.2	1	41.2	4	43.2	13	45.2	4
39.3	2	41.3	9	43.	12	45.3	1
39.4	1	41.4	6	43.4	14	45.4	4
39.5	..	41.5	11	43.5	12	45.5	2
39.6	2	41.6	13	43.6	13	45.6	1
39.7	1	41.7	14	43.7	10	45.7	1
39.8	1	41.8	17	43.8	10	45.8	3
39.9	2	41.9	2	43.9	9	45.9	5
40.0	5	42.0	17	44.0	11		
40.1	..	42.1	13	44.1	13		
40.2	4	42.2	15	44.2	9		
40.3	3	42.3	20	44.3	7		
40.4	7	42.4	11	44.4	7		
40.5	5	42.5	16	44.5	11		
40.6	4	42.6	14	44.6	9		
40.7	5	42.7	9	44.7	4		
40.8	3	42.8	21	44.8	5		
40.9	5	42.9	19	44.9	5		

[1] This figure includes 49 samples from W. G. Tice, State board of health, Trenton, N. J. (U. S. Bur. Chem. Bull., 132, p. 130).

TABLE 6.

Refractions of sour serum on 504 samples of milk of known purity.

READING	NUMBER OF SAMPLES	READING	NUMBER OF SAMPLES	READING	NUMBER OF SAMPLES	READING	NUMBER OF SAMPLES
38.3	4	40.3	11	42.3	9	44.3	1
38.4	2	40.4	9	42.4	19	44.4	3
38.5	2	40.5	6	42.5	6	44.5	3
38.6	3	40.6	8	42.6	7	44.6	1
38.7	2	40.7	9	42.7	10	44.7	2
38.8	2	40.8	10	42.8	10	44.8	1
38.9	1	40.9	6	42.9	5	44.9	2
39.0	9	41.0	18	43.0	14	45.0	2
39.1	5	41.1	10	43.1	8	45.1	1
39.2	6	41.2	11	43.2	8	45.2	1
39.3	2	41.3	15	43.3	5	45.3	..
39.4	9	41.4	10	43.4	7	45.4	..
39.5	7	41.5	17	43.5	10	45.5	1
39.6	2	41.6	10	43.6	5	45.6	..
39.7	4	41.7	13	43.7	11	45.7	1
39.8	5	41.8	13	43.8	4	45.8	..
39.9	8	41.9	11	43.9	3	45.9	..
40.0	11	42.0	15	44.0	4		
40.1	3	42.1	18	44.1	2		
40.2	8	42.2	19	44.2	4		

TABLE 7.

Relation between refractions of copper sulphate and acetic acid serum on 325 samples of milk of known purity.

REFRACTION OF ACETIC SERUM	REFRACTION OF COPPER SERUM														PER CENT OF SAMPLES
Reading at 20°C.	36.0–36.2	36.3–36.5	36.6–36.8	36.9–37.1	37.2–37.4	37.5–37.7	37.8–38.0	38.1–38.3	38.4–38.6	38.7–38.9	39.0–39.2	39.3–39.5	39.6–39.8	Totals	
	Number of samples														
39.0–39.5	1[1]	..	1	..	..	..	..	..	..	..	..	..	..	2	0.6
39.6–40.1	3	..	1	..	..	..	..	..	..	..	..	..	..	4	1.2
40.2–40.7	1	6	3	3	2	..	..	..	..	..	..	..	..	15	4.6
40.8–41.3	..	5	5	10	6	1	..	..	..	..	..	..	..	27	8.3
41.4–41.9	..	..	3	4	9	9	5	2	1	..	..	..	1	34	10.5
42.0–42.5	..	..	3	1	6	10	22	7	2	..	..	..	..	51	15.7
42.6–43.1	..	..	..	3	5	10	25	14	8	2	2	..	..	69	21.3
43.2–43.7	..	2	..	..	..	1	6	10	11	4	8	..	..	42	12.9
43.8–44.3	..	..	..	..	1	4	5	9	9	6	4	1	..	39	12.0
44.4–44.9	..	..	..	1	1	..	2	4	7	6	5	1	..	27	8.3
45.0–45.5	..	..	..	..	1	..	1	..	..	5	3	2	1	13	4.0
45.6–46.1	..	..	..	..	..	..	..	..	..	..	1	..	1	2	0.6
Totals........................	5	13	16	22	31	35	66	46	38	23	23	4	3	325	
Per cent of samples........	1.5	4.0	4.9	6.8	9.5	10.8	20.3	14.2	11.7	7.1	7.1	1.2	0.9		100

[1] Lowest sample: copper serum 36.1; acetic serum 39.0.

Tables 7, 8 and 9, present the relations existing between (a) the copper and acetic serum, (b) the copper and sour serum, and (c) the acetic and sour serum. In Table 7 the range of the acetic refractions is twice as large as that of the copper refraction, so that the ratio of 3 copper refractions to the copper limit is the same as 6 acetic refractions to the acetic limits. The table has, therefore, been arranged in that manner. In Table 8 the ratio is 4 copper refractions to 9 sour serum refractions, while in Table 9, 6 sour serum refractions equal 7 acetic refractions.

In Table 7, 20 per cent of the copper samples refracted between 37.8 and 38.0 and 21 per cent of the acetic between 42.6 and 43.1. Twenty-five samples, or 7.7 per cent of the total samples, had both copper and acetic refractions within the above-stated limits. From this point the relationship between the 2 serums is fairly uniform.

TABLE 8.

Relation between refractions of copper and sour serum on 364 samples of milk of known purity.

REFRACTION OF SOUR SERUM	REFRACTION OF COPPER SERUM											PER CENT OF SAMPLES
Reading at 20°C.	36.0–36.3	36.4–36.7	36.8–37.1	37.2–37.5	37.6–37.9	38.0–38.3	38.4–38.7	38.8–39.1	39.2–39.5	39.6–39.9.	Totals	
	Number ot samples											
37.9–38.7	4[1]	4	..	..	..	..	..	..	..	..	8	2.2
38.8–39.6	5	13	6	3	1	..	..	..	..	..	28	7.7
39.7–40.5	6	8	20	6	4	2	..	..	..	..	46	12.6
40.6–41.4	4	2	7	23	18	11	4	2	..	..	71	19.5
41.5–42.3	..	2	4	11	21	32	19	7	..	..	96	26.4
42.4–43.2	..	..	1	3	9	20	17	13	4	..	67	18.4
43.3–44.1	1	..	1	..	..	7	11	5	4	2	31	8.5
44.2–45.0	..	1	1	..	..	2	..	6	1	2	13	3.6
45.1–45.9	..	..	..	..	1	1	..	..	..	2	4	1.1
Totals.........	20	30	40	46	54	75	51	33	9	6	364	
Per cent of samples......	5.5	8.2	11.0	12.7	14.8	20.5	14.0	9.1	2.5	1.6		100

[1] Lowest sample: sour serum 38.3; copper serum 36.0.

In Table 8, 20 per cent of the copper refractions were between 38.0 and 38.3, and 26 per cent of the sour refractions between 41.5 and 42.3; 32 samples, or 8.7 per cent of the total, gave copper and sour serum readings between the above-stated limits. The relationship here is not so uniform as in the case of the copper and acetic comparison, but this is due, undoubtedly, to the unusual forms of fermentation occurring during the souring of the milk.

In Table 9, 19.8 per cent of the acetic refractions were between 43.0 and 43.5; 22.1 per cent of the sour refractions between 41.5 and 42.1; 10 samples, or 6 per cent of the total, gave sour and acetic readings between

TABLE 9.

Relation between refractions of acetic and sour serums on 167 samples of milk of known purity.

REFRACTION OF SOUR SERUM	REFRACTION OF ACETIC SERUM										TOTALS	PER CENT OF SAMPLES
Reading at 20°C.	40.0–40.5	40.6–41.1	41.2–41.7	41.8–42.3	42.4–42.9	43.0–43.5	43.6–44.1	44.2–44.7	44.8–45.3	45.4–45.9		
	Number of samples											
38.0–38.6	1[1]	...	....	....	....	1	....	...	...	...	2	1.2
38.7–39.3	1	...	1	....	....	2	....	...	...	1	5	3.0
39.4–40.0	1	1	3	....	1	....	....	...	...	...	6	3.6
40.1–40.7	5	1	6	6	1	2	1	...	...	...	22	13.2
40.8–41.4	...	3	4	4	6	4	1	2	...	...	24	14.4
41.5–42.1	...	1	4	13	7	7	3	...	2	...	37	22.1
42.2–42.8	...	...	....	6	5	10	4	4	...	...	29	17.3
42.9–43.5	...	...	....	....	4	3	7	4	2	2	22	13.2
43.6–44.2	...	...	1	2	1	3	3	...	2	1	13	7.8
44.3–44.9	...	...	....	1	....	1	1	1	1	2	7	4.2
Totals.......	8	6	19	32	25	33	20	11	7	6	167	....
Per cent of samples......	4.8	3.6	11.3	19.1	15.0	19.8	12.0	6.6	4.2	3.6	...	100

[1] Lowest sample: sour serum 38.3; acetic serum 40.1.

the above-stated limits. In general, the sour serum refracts lower than the acetic. The largest number of samples (13 or 7.8 per cent) with acetic refractions of 41.8 and 42.3 and sour serum refractions of 41.5 to 42.1 would, without doubt, not occur at this point if a larger number of samples had been examined.

During the past three years determinations have been made of the ash of the sour serum.

> Allow the milk to sour spontaneously, pipette 25 cc. of the clear serum into a flat-bottomed platinum dish and evaporate to dryness over the water-bath. Heat cautiously the contents of the dish over a small flame (to avoid sputtering) until charred. Perform the final heating in the electric muffle (with a pyrometer connected) at a heat not greater than 500°C. or 900°F. A white ash is almost invariably obtained after once leaching.

In the original article as described by A. Burr and F. M. Berberich,[1] the authors have outlined the following method:

> Measure 50 cc. of the serum into a platinum dish, evaporate to dryness and carbonize over a low flame. Extract the char with hot water, burn the insoluble residue, add the solution to this ash, evaporate to dryness, ignite at a low temperature and weigh.

Experience has shown, however, that with the exercise of care and an accurate pyrometer the former stated method is as accurate as the latter and involves less manipulation.

[1] *Chem. Ztg.*, 1908, **32**: 617.

Table 10 gives the ash of the sour serum, expressed in grams per 100 cc., of 371 samples of known purity milk.

TABLE 10.

Ash of the sour serum on 371 samples of milk of known purity.
(Expressed in grams per 100 cc.)

ASH	NUMBER OF SAMPLES	ASH	NUMBER OF SAMPLES	ASH	NUMBER OF SAMPLES	ASH	NUMBER OF SAMPLES
0.730–0.734	22	0.790–0.794	20	0.850–0.854	2	0.910–0.914	..
0.735–0.739	8	0.795–0.799	16	0.855–0.859	4	0.915–0.919	..
0.740–0.744	26	0.800–0.804	22	0.860–0.864	6	0.920–0.924	..
0.745–0.749	12	0.805–0.809	13	0.865–0.869	6	0.925–0.929	..
0.750–0.754	14	0.810–0.814	19	0.870–0.874	3	0.930–0.934	1
0.755–0.759	10	0.815–0.819	8	0.875–0.879	1	0.935–0.939	..
0.760–0.764	26	0.820–0.824	17	0.880–0.884	2	0.940–0.944	2
0.765–0.769	11	0.825–0.829	4	0.885–0.889	3		
0.770–0.774	16	0.830–0.834	4	0.890–0.894	1		
0.775–0.779	9	0.835–0.839	2	0.895–0.899	1		
0.780–0.784	28	0.840–0.844	5	0.900–0.904	2		
0.785–0.789	17	0.845–0.849	7	0.905–0.909	1		

As will be noticed, practically the same percentage of samples, 18 per cent, was found with ashes between the following limits: 0.730 and 0.749; 0.750 and 0.769; 0.770 and 0.789; 0.790 and 0.810; or, in 72 per cent of the cases the limits of the ash extended from 0.730–0.810. Thirteen per cent of the samples had ashes from 0.810–0.830. Five per cent of the ashes were from 0.830–0.850 and 5 per cent from 0.850–0.870, making 10 per cent between 0.830 and 0.870. The remaining 5 per cent gave figures above 0.830 which were distributed as extensively as the first 72 per cent.

TABLE 11.

Composition of 67 samples of herd milk.
(Arranged in order of Total Solids.)

TOTAL SOLIDS	FAT	SOLIDS NOT FAT	COPPER REFRACTION	ACETIC REFRACTION	SOUR SERUM REFRACTION	SOUR SERUM ASH
per cent	*per cent*	*per cent*	*reading*	*reading*	*reading*	*grams per 100 cc.*
15.32	5.30	10.02	38.2	41.4	....	.....
14.80	5.90	8.90	38.5	....	43.3	0.964
14.57	5.40	9.17	38.5	44.1	....	.....
14.40	5.00	9.40	38.6	44.6	....	.....
14.19	5.00	9.19	38.3	....	42.7	0.792
13.84	4.50	9.34	38.0	....	41.9	0.808
13.73	4.60	9.13	37.7	42.4	41.8	0.806
13.68	5.10	8.58	38.8	....	42.7	0.960
13.68	4.80	8.88	37.8	41.6	42.1	0.796
13.60	4.45	9.15	38.7	45.4	....	.....

TABLE 11.—Continued.

TOTAL SOLIDS	FAT	SOLIDS NOT FAT	COPPER REFRACTION	ACETIC REFRACTION	SOUR SERUM REFRACTION	SOUR SERUM ASH
per cent	*per cent*	*per cent*	*reading*	*reading*	*reading*	*grams per 100 cc.*
13.48	4.40	9.08	38.0	42.3	41.9	0.788
13.48	4.10	9.38	37.8	42.3	42.4	0.810
13.46	4.50	8.96	38.0	42.4	42.0	0.809
13.45	4.35	9.10	....	43.6	42.9	.....
13.44	4.10	9.34	38.2	43.4	....	.....
13.40	4.20	9.20	38.5	....	42.2	0.780
13.35	4.00	9.35	....	44.3	42.8	.....
13.34	4.30	9.04	38.3	....	42.2	0.800
13.32	5.00	8.32	38.0	....	43.5	0.800
13.28	4.50	8.78	38.2	43.1	....	.....
13.27	4.40	8.87	37.7	....	....	.....
13.08	4.75	8.33	37.7	42.2	....	.....
13.08	4.30	8.78	38.2	....	42.1	0.740
13.08	4.10	8.98	37.7	....	41.3	0.788
13.06	4.05	9.01	38.1	42.3	42.6	0.812
12.97	4.40	8.57	38.0	....	42.3	0.760
12.96	4.00	8.96	....	42.1	41.0	.....
12.95	4.00	8.95	37.7	42.0	....	.....
12.92	4.20	8.72	38.0	42.0	41.6	0.796
12.88	4.00	8.88	37.5	....	41.4	0.771
12.80	3.80	9.00	37.9	....	42.3	0.776
12.76	4.40	8.36	....	42.5	41.5	.....
12.76	3.80	8.96	37.8	....	....	.....
12.73	3.80	8.93	38.0	....	41.4	0.800
12.72	3.90	8.82	38.8	....	43.0	0.792
12.67	3.90	8.77	....	43.5	....	.....
12.64	3.60	9.04	38.2	....	43.0	0.800
12.62	3.85	8.77	37.2	....	....	.....
12.59	3.70	8.89	38.3	....	41.4	0.768
12.58	3.60	8.98	38.0	....	42.0	0.812
12.57	4.00	8.57	38.1	43.1	....	.....
12.57	4.00	8.57	37.2	....	41.5	0.766
12.56	4.20	8.36	37.9	43.1	....	.....
12.55	3.65	8.90	....	43.4	....	....
12.53	3.70	8.83	37.7	....	41.5	0.808
12.52	4.00	8.52	37.2	41.0	....	.....
12.49	3.65	8.84	....	43.0	42.3	.....
12.45	3.60	8.85	38.3	....	....	.....
12.44	3.70	8.74	38.0	....	42.0	0.750
12.36	3.90	8.46	38.0	40.2	40.3	0.794
12.26	3.70	8.56	37.5	....	40.7	0.820
12.25	3.70	8.55	....	42.9	....	.....
12.24	3.70	8.54	....	41.3	....	.....
12.22	3.50	8.72	....	43.9	42.6	.....
12.18	3.80	8.38	37.9	42.0	....	.....
12.07	3.40	8.67	37.1	....	....	.....
12.03	3.60	8.43	38.5	42.6	....	.....
12.02	3.40	8.62	37.7	41.8	41.9	0.764
12.00	3.90	8.10	36.7	....	40.0	0.788
11.96	3.35	8.61	37.2	42.2	....	.....

TABLE 11.—Continued.

TOTAL SOLIDS	FAT	SOLIDS NOT FAT	COPPER REFRACTION	ACETIC REFRACTION	SOUR SERUM REFRACTION	SOUR SERUM ASH
per cent	*per cent*	*per cent*	*reading*	*reading*	*reading*	*grams per 100 cc.*
11.84	3.50	8.34	37.7	....	....	.....
11.80	3.55	8.25	....	42.5	41.0	.....
11.74	3.40	8.34	37.0	....	39.6	0.780
11.74	3.30	8.44	37.4	....	40.3	0.786
11.28	3.20	8.08	37.1	....	39.5	0.752
11.25	3.60	7.65	36.6	....	....	.....
11.06	3.30	7.76	36.6	....	....	.....
Total samples 67	67	67	56	34	42	34
Maximum 15.32	5.90	10.02	38.8	45.4	43.5	0.964
Minimum 11.06	3.20	7.65	36.6	40.2	39.5	0.740
Average 12.83	4.06	8.77	37.9	42.7	41.7	0.798

The above 67 herds are representative of all the breeds mentioned on page 9 of this report.

Here, as would be expected in herd milk, the copper, acetic and sour serum refractions and the sour serum ash figures are higher than the minimum obtained from individual cows. The lowest figure approaching nearest to the minimum copper limit (36.0) is 36.6; to the minimum acetic limit (39.0) is 40.2; and to the sour serum limit (38.3) is 39.5; and the nearest approach to the sour serum ash limit (0.730) is 0.740. The sour serum ash figures are high, not only in the greater majority of cases, but particularly wherever there is a tendency for the copper, acetic or sour serums to refract lower than the average figures given above.

COMMENTS OF ANALYSTS ON THE COPPER METHOD.

A. G. Woodman: My work on milk has been almost entirely on isolated samples for educational purposes, and practically no samples of undoubted authenticity, so I can give you no figures that would be of use to you. Personally, I much prefer the copper method to the acetic acid as we get a clear serum with much less trouble, and I hope that the copper method will be adopted by the association.

Wm. Frear: In Pennsylvania we are using the Woodman acetic acid method, not because of any particular preference, except that the records from its use give a wider range for comparison than are available from any other copper sulphate method.

W. C. Woodward: Owing to our extremely limited laboratory facilities and the large number of analyses made, we have accomplished little research work of any kind, and therefore have no data available on the subject. The laboratory has, however, tested this method to some extent, and although comparing favorably

TABLE 12.

Composition of a sample of milk systematically watered.[1]

PER CENT ADDED WATER	PER CENT TOTAL SOLIDS	PER CENT FAT	PER CENT ASH	ACETIC SERUM					COPPER SERUM					SOUR SERUM			
				Re-fraction at 20°C.	Sp.gr. at $\frac{20^\circ C.}{4^\circ}$	Per cent Solids	$\frac{n^2-1}{n^2+2}\cdot\frac{1}{d}$	$\frac{n-n^1}{C}$	Re-fraction at 20°C.	Sp. gr. at $\frac{20^\circ C.}{4^\circ}$	Per cent Solids	$\frac{n^2-1}{n^2+2}\cdot\frac{1}{d}$	$\frac{n-n^1}{C}$	Refraction at 20°C.	Sp. gr. at $\frac{20^\circ C.}{4^\circ}$	Per cent Ash	$\frac{n^2-1}{n^2+2}\cdot\frac{1}{d}$
0	12.19	3.60	0.71	41.4	1.0269	6.49	0.20589	0.00159	37.3	1.0261	5.61	0.20523	0.00153	39.9	1.0254	0.740	0.20588
10	10.97	3.24	0.64	38.6	1.0241	5.89	0.20589	0.00157	35.4	1.0243	5.15	0.20518	0.00156	37.5	1.0224	0.666	0.20601
20	9.75	2.88	0.57	35.6	1.0208	5.16	0.20592	0.00157	33.5	1.0223	4.62	0.20518	0.00158	34.7	1.0197	0.572	0.20597
30	8.53	2.50	0.50	33.0	1.0181	4.45	0.20592	0.00160	31.5	1.0204	4.14	0.20516	0.00158	32.0	1.0169	0.520	0.20597
40	7.31	2.16	0.43	30.1	1.0151	3.85	0.20594	0.00156	29.5	1.0118	3.65	0.20507	0.00158	29.4	1.0142	0.428	0.20597

n = Refractive index (not scale reading) of serum; n^1 = Refractive index of water; n^2 = square of refractive index of serum; d = Specific gravity; c = Concentration as per cent.

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[1] Proc. 8th Intern. Cong. Appl. Chem., 1912, 1: 311.

with the acetic acid method as to efficiency we prefer the latter as the serum is uncolored, the line of refraction more clearly defined, and therefore much more easily and accurately read, especially in sera which are not absolutely clear.

H. H. Hanson: Although we are using the copper sulphate method in our laboratory, we have no determinations upon milk of known purity.

H. Louis Jackson: I abandoned the acetic acid method of preparing the serum several years ago and much prefer the copper method. I feel that it should most certainly be made at least an optional provisional method.

E. M. Bailey: I made the suggestion in regard to the copper serum at the meeting last year because so much data had already been collected and because so many analysts seemed to be using it in preference to the acetic acid serum. Mr. Conn of our State board of health has used it for some years and his experience coincides with mine as regards range of readings. I have never found a milk which I thought was straight which read below 36.

Lythgoe[1] has criticized the copper method, and subsequently Ackermann,[2] on account of the dilution necessary in the preparation of the serum, but this is offset by less variation in the serum from different samples. Another disadvantage is that it is not possible to use the values of the ash of the copper serum in detecting added water because the amount of copper in the serum is higher in watered milk than in unwatered milk, and consequently the ash in the serum of watered samples is but little less than in the serum of the original milk before watering.

RECOMMENDATIONS.

It is recommended—

(1) That the following be adopted as auxiliary provisional methods:

DETECTION OF ADDED WATER.

(*a*) *Copper serum.*[3]—To 1 volume of copper sulphate solution ($72\frac{1}{2}$ grams of copper sulphate per liter, adjusted if necessary to read 36.0 at 20°C. on the scale of the Zeiss immersion refractometer, or, to a specific gravity of 1.0443 at $\frac{20°C.}{4°}$) add 4 volumes of milk. Shake well and filter. Determine the refractive index of the clear serum by means of the Zeiss immersion refractometer. A reading below 36.0 indicates added water.

(*b*) *Sour serum.*[4]—Allow the milk to sour spontaneously, filter and determine the index of refraction of the clear serum by means of the Zeiss immersion refractometer. A reading below 38.0 indicates added water.

(*c*) *Ash of sour serum.*[5]—Allow the milk to sour spontaneously and filter. Transfer 25 cc. of this serum to a flat-bottomed platinum dish and evaporate to dryness over the water-bath. Heat the contents of the dish over a small flame (to avoid sputtering) until charred. Place the dish in an electrically heated muffle (with pyrometer connected) and ash at a temperature not greater than 500°C. or 900°F. Cool and weigh. Express results as grams per 100 cc. An ash below 0.730 indicates added water.

(*d*) *Ash of acetic serum.*—Transfer 25 cc. of the serum to a flat-bottomed platinum dish and proceed as directed under (*c*). An ash figure below 0.715 grams per 100 cc. indicates added water.

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[1] Proc. 8th Intern. Cong. of Appl. Chem., 1912, **1**: 308.

[2] *Z. Nahr. Genussm.*, 1912, **24**: 612.

[3] Mass. State Board of Health Rep., 1908, p. 594.

[4] *Z. offent. Chem.*, 1903, **9**: 173.

[5] *Chem. Ztg.*, 1908, **32**: 617.

It is recommended in conjunction with the copper, acetic or sour serum refraction method that the determination of the ash of the sour serum or of the acetic serum be made in all cases where the indices of refraction fall below the minimum limit so as to eliminate all possibility of the sample being abnormal milk from a sick cow. The acetic serum ash multiplied by the factor 1.021 equals the sour serum ash (dilution of the acetic serum being 2 per cent).

(2) That further study be made on the Harding-Parkin[1] method for fat determination in comparison with the present official and provisional methods.

(3) That in U. S. Bur. Chem. Bull. 107, Rev., p. 122, after the paragraph on Cream, the following paragraph be inserted under the heading "Condensed Milk (Unsweetened)": "Dilute 40 grams of the homogeneous material with 60 grams of distilled water, proceed as directed under 'Milk' and correct the results for dilution"; and the word "Sweetened" be inserted before the word "Condensed" in the subsequent heading.

(4) That further study be given to enzyme reactions of milk.

REPORT ON SEPARATION OF NITROGENOUS SUBSTANCES.

By A. D. Emmett (Agricultural Experiment Station, Urbana, Ill.), *Associate Referee.*[2]

During the past year samples with detailed outline of directions were sent out to five laboratories. Owing to pressure of other duties, only two reports were received, and but one of these was complete. As a result, the referee feels that the data given in the following tables are inadequate for definite deductions, and suggests that the work be continued along these lines another year.

It is suggested that the Folin method for creatin and creatinin determinations, as outlined for meats and meat extracts, be made official but that the standard used be the usual potassium dichromate, until more work has been done on obtaining perfectly satisfactory salts of creatinin.

It would appear from the ammonia determinations (Table 3) that the use of sodium hydroxid has a definite and distinct effect in comparison with potassium carbonate. Whether this additional liberation of ammonia is due solely to the breaking up of the ammonium magnesium phosphate is not as yet clear from these data. This point should be studied more thoroughly.

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[1] *J. Ind. Eng. Chem.*, 1913, **5**: 131.

[2] Read by P. F. Trowbridge.

TABLE 1.

Percentage of creatin and creatinin in meat extracts and meats (Folin method).

SAMPLE	CREATIN			CREATININ		
	F. C. Cook	C. R. Moulton	A. D. Emmett	F. C. Cook	C. R. Moulton	A. D. Emmett
Meat Extract:						
1	3.31	2.95	2.82	4.11	4.41	4.66
2	3.07	2.96	2.99	4.13	4.47	4.46
3	3.09	3.69[1]	....	4.19	4.33	4.59
Average.........	3.16	2.95	2.90	4.14	4.41	4.57
Dried Meat:						
1	1.30	1.84	1.23	....	....	....
2	1.31	1.87	1.25	....	....	....
3	1.40	1.83	....	....	....	....
Average.........	1.33	1.85	1.24	....	....	....

[1] Omitted from average.

TABLE 2.

Percentage of amino nitrogen in meat extracts and meats.

SAMPLE	VAN SLYKE METHOD		KOBER METHOD
	F. C. Cook	A. D. Emmett	F. C. Cook
Meat Extract:			
1	1.16	0.70	0.41
2	1.22	0.50	0.47
Dried Meat:			
1	0.22	0.59	0.33
2	0.25	0.70	0.30

TABLE 3.

Percentage of ammonia nitrogen in meat extracts and meats.

SAMPLE	F. C. COOK AND J. B. WILSON			A. D. EMMETT		
	Aeration method		Magnesium oxid method	Aeration method		Magnesium oxid method
	Folin (K_2CO_3)	Steel-Gies (NaOH)		Folin (K_2CO_3)	Steel-Gies (NaOH)	
Meat Extract:						
1	0.402	0.498	0.468	0.644	0.712	0.479
2	0.393	0.458	0.485	0.633	0.702	0.481
3	0.332	0.361	0.507	0.612	0.713	.....
Average.........	0.376	0.439	0.487	0.633	0.709	0.480
Dried Meat:						
1	0.055	0.197	0.225	0.086	0.257	0.111
2	0.055	0.203	0.225	0.091	0.272	0.147
3	0.053	.....	.....	.....	.....	.....
Average.........	0.054	0.200	0.225	0.088	0.264	0.129

TABLE 4.

Percentage rate of liberation of ammonia by aeration methods.

(By A. D. Emmett.)

SAMPLE	FOLIN METHOD					STEEL-GIES METHOD				
	1 hour	20 minutes	20 minutes	1 hour	Total $2\frac{2}{3}$ hours	1 hour	20 minutes	20 minutes	1 hour	Total $2\frac{2}{3}$ hours
BEEF EXTRACT:										
1	0.491	0.069	0.042	0.042	0.644	0.513	0.081	0.052	0.066	0.712
2	0.466	0.095	0.036	0.036	0.633	0.511	0.077	0.049	0.065	0.702
3	0.482	0.057	0.040	0.033	0.612	0.525	0.082	0.045	0.061	0.713
Average.......	0.483	0.074	0.039	0.037	0.633	0.516	0.080	0.049	0.064	0.709
Percentage....	76.3	11.7	6.2	5.8	100.0	72.8	11.3	6.9	9.0	10.00
Percentage increase due to Sugiura-Gies method	6.4	0.7	20.4	41.2	10.7	......	......	......	......	......

PLAN FOR COÖPERATIVE WORK.

It is suggested that the work be undertaken along the following lines: (a) Further study of the determination of creatin and creatinin nitrogen; (b) determination of amino nitrogen (amino acids); and (c) ammonia nitrogen.

The determinations should be made in triplicate if possible, that is, on three portions of the original sample.

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SAMPLES.

The samples to be studied consist of desiccated lean beef and beef extract.

The meat was first thoroughly dried by a special procedure at room temperature, then in vacuo. Care should be taken to make all weighings by difference and to keep the bottle covered when not in use. If possible, keep the samples in a refrigerator until ready to analyze.

Before making any weighings mix carefully each sample. In the case of the meat, rotate the bottle in various directions and in the case of the beef extract, warm the jar on the edge of the steam-bath for 5–10 minutes, then stir and transfer a portion to a weighing bottle and allow it to cool for weighing.

PREPARATION OF BEEF EXTRACT SOLUTIONS.

Weigh off (by difference) about 5 grams of the sample into 150 cc. beakers. Dissolve in cold (20°–25°C.) ammonia-free distilled water. Transfer the solution carefully to a 250 cc. measuring flask. Dilute to the mark and mix thoroughly.

Take in duplicate the following quantities, mixing each time before measuring: (a) creatinin, 5 cc.; (b) creatin, 20 cc.; (c) amino acids, 20–40 cc.; (d) ammonia (aeration method), 25 cc.; (magnesium oxid method), 50 cc.

I. CREATIN AND CREATININ.[1]

Solutions and reagents.—Ten per cent solution of sodium hydroxid, carbonate-free; picric acid, saturated solution, 1.2 per cent; potassium dichromate, and standard creatin solution.

Apparatus.—Two hundred cc. volumetric flasks; 100 cc. volumetric flasks; autoclave; colorimeter, Duboscq if possible.

(a) CREATIN.

Determination.

(*a*) *Meats.*—Transfer 5 grams of the fresh meat, or 2 grams of the dried meat (weighed by difference), to a 200 cc. Erlenmeyer flask. Add 100 cc. of N/2 sulphuric acid. Cover the flask with tinfoil or a crucible lid and heat in the autoclave at 130°–135°C. for 30 minutes. Allow the temperature of the autoclave to fall and remove the flask. Cool it under running water and transfer the solution to a 200 cc. volumetric flask, using cold distilled water to aid in the transfer. Shake, to break up the flocculent skeletons of the tissue, dilute to the mark, and mix thoroughly.

Pour the solution on a quantitative filter, collecting filtrate in a dry flask or beaker. Titrate 10 cc. with 10 per cent sodium hydrate, using phenolphthalein as indicator. Measure 10 cc. into a 100 cc. measuring flask, add 20 cc. of the saturated picric acid and "enough 10 per cent sodium hydrate solution to give 1.5 cc. over and above that required for the neutralization of the sulphuric acid"; let stand 10 minutes, dilute to the mark, mix, and read in the colorimeter.

Use the standard creatinin-zinc chlorid solution sent out with the sample (1.389 grams per liter or 1 mg. of creatin per cc.). Set the standard at 10 mm.

(*b*) *Beef extract.*—Transfer 20 cc. of the beef extract to a 200 cc. volumetric flask add 100 cc. N/2 sulphuric acid and proceed exactly as directed under (*a*) for creatin in meat. Set the standard at 15 mm.

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[1] Reprints by Folin accompanied instructions to collaborators.

(b) CREATININ.

Determination.

Beef extract.—Transfer 5 cc. of the beef extract solution to a 100 cc. volumetric flask, add 20 cc. of the saturated picric acid and then 1.5 cc. of the 10 per cent sodium hydrate solution. Mix and let stand for 10 minutes, after which dilute to the mark and mix and read in colorimeter. Set the standard at 20 mm. If the readings are too low adjust the standard at 15 mm. The creatin determination in the beef extract includes the creatinin and proper corrections should be made for obtaining the actual creatin, namely, subtract the preformed creatinin from the total creatinin and multiply the difference by 1.16.

NOTE.—In all of the work on creatin and creatinin if the readings are less than two-thirds or more than one and one-half those of the standard, the determinations should be repeated with more or less of the solution.

II. AMMONIA.

(a) AERATION METHOD.[1]

Solutions and reagents.—Saturated solution of potassium oxalate (325 grams per liter), potassium carbonate (817 grams per liter), sodium hydrate (506 grams per liter), and kerosene to retard foaming. Sulphuric acid (1 to 4) for wash bottle. Seventieth-normal acid and seventieth-normal alkali. Alizarin red as indicator.

Apparatus.—Test tubes, 10 x 1 inch, or lipless cylinder 8 x $1\frac{1}{4}$ inches; 150 cc. Florence flasks; rubber disk to prevent splashing into receiving flask; suction pump. (See *J. Biol. Chem.*, 1912, **11**: 499).

Determination.

Transfer about 5 grams of dried meat or 10 grams of fresh meat (weighed by difference) or 25 cc. of the beef extract solution to a test tube or cylinder. Add 3 to 5 grams of thoroughly washed sand, 10 cc. of distilled ammonia-free water and mix with a glass rod, then add 10 cc. more of water and stir. Remove the rod, washing it down with 5 cc. of distilled water and 10 cc. of the oxalate solution. Add 3 to 4 drops of kerosene and then 5 cc. either of the potassium carbonate or sodium hydroxid solution. At once connect up the apparatus, and start the suction or pressure, gently increasing it during the first minute or two. Allow the air current to run at a high rate for 45 minutes. Then replace the receiving flask with a second one which contains 5 cc. of the standard acid. Continue the aeration for 20 minutes longer. Replace the second receiving flask with another containing 5 cc. of acid and let the operation continue for 20 minutes. Replace the third flask and continue the aeration for an hour. Titrate each distillate with seventieth-normal sodium hydroxid, using 5 drops of alizarin red.

NOTE.—Make a comparison of the effect of using potassium carbonate and sodium hydrate.

(b) MAGNESIUM OXID METHOD.

(See U. S. Bur. Chem. Bull. 107, Rev., p. 9.) Use 50 cc. of the beef extract solution.

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[1] Reprints by Folin and Farmer and Folin and Macallum, and Shulansky and Gies accompanied instructions to collaborators.

III. AMINO ACID NITROGEN.

(a) VAN SLYKE METHOD.

Solutions and reagents.—Alkaline permanganate, 50 grams of potassium permanganate and 25 grams of potassium hydroxid per liter; sodium nitrite, 300 grams per liter; glacial acetic acid; caprylic alcohol; sulphuric acid, 1 per cent solution; 25 per cent (316 grams per liter) sodium hydrate solution; 50 per cent acetic acid solution; and 95 and 80 per cent ethyl alcohol.

Apparatus.—A special apparatus is required for this method. This can be procured from Emil Greiner, 45 Cliff Street, New York City. The micro-apparatus is preferable. Mr. Van Slyke suggests that in ordering, it be specified that a 2 cc. cylinder be attached to either apparatus for the caprylic alcohol. It is sometimes necessary in working with tissue to add the alcohol quickly to stop foaming.

Five hundred and 1000 cc. double-necked distilling flasks; and 25 cc. volumetric flasks.

Determination.

(a) *Meats.*—Take in duplicate 10 grams (dried meat) or 40 grams (fresh meat), weighed by difference. Transfer it to 200 cc. Erlenmeyer flasks. Add 100 cc. of boiling water to which 1 cc. of 50 per cent acetic acid per liter is added. Stir and digest for 15 minutes at 100°C. Decant the solution through a glass wool filter, taking care to prevent the particles of meat from passing over. Add to the residue 75 cc. of the hot acid water and heat for 5 minutes. Decant the solution on the same filter. Repeat the extraction 4 more times. Transfer the extract to a liter double-necked distilling flask and concentrate under diminished pressure until the volume of the solution is about 20 cc. Allow the distillation to continue as rapidly as possible without loss of solution by foaming. Transfer the solution to a 250 cc. volumetric flask, using 2 portions of about 5 cc. each of water, then 3 portions of 10 cc. each of 95 per cent alcohol, dilute to the mark with 95 per cent alcohol, mix thoroughly and measure off 100 cc. for the Kober-Sugiura method, and let the remainder stand overnight. Filter the alcoholic solution through a folded filter into a liter distilling flask, wash with 80 per cent alcohol. Add, drop by drop, to the filtrate enough 25 per cent sodium hydrate to make the filtrate alkaline to phenolphthalein. Again concentrate under diminished pressure to remove both ammonia and alcohol. When the volume of the concentrating solution has reached 10 or 20 cc. the distillation is interrupted and enough 50 per cent acetic acid added to acidify the solution. About 50 cc. of water are also added, in order to insure that the last traces of alcohol are driven off, and the concentration continued. Evaporate to 10-15 cc. and transfer cautiously with water to a 25 cc. volumetric flask. Dilute to the mark and mix.

Take 10 cc. portions of this solution for the large apparatus and 2 cc. portions for the micro-apparatus. Continue the procedure exactly as described by Van Slyke (*J. Biol. Chem.* 1911, **9**: 189; 1912, **12**: 279; and 1914, **16**: 121.) The time for the reaction should be maintained carefully; for temperatures 15°-20°C., 4-5 minutes; for 20°-25°C., 3 minutes; and for 25°-30°C., 2½ minutes. In calculating the results consult the table given in the *J. Biol. Chem.*, 1912, **12**: 284.

(b) *Beef extract.*—Take 25-40 cc. of the original solution and transfer it to a 250 cc. volumetric flask, dilute to the mark with 95 per cent alcohol. Measure out *100 cc.* for the Kober-Sugiura method, and let the remainder stand overnight. Filter on a folded filter and proceed exactly as directed under (*a*) for this stage of the work.

(b) KOBER AND SUGIURA METHOD.[1]

Solutions and reagents.—Cupric hydroxid; anhydrous cupric chlorid solution, 5 per cent; barium hydroxid, saturated solution; sodium hydroxid, normal solution; alkaline sodium boric acid solution; hydrochloric acid, tenth-normal solution; glacial acetic acid; phenolphthalein, 0.10 per cent solution; four-thousandths-normal sodium thiosulphate solution; starch solution; potassium iodid crystals; alcoholic solution zinc chlorid, saturated.

In the preparation of the cupric hydroxid and "buffer" solution the details should be followed exactly as given by the authors.

Determination.

(*a*) *Meats.*—Take the 100 cc. of the alcoholic solution prepared by the Van Slyke method as directed under (*a*), transfer to a 100 cc. volumetric flask and add a few drops of the alcoholic zinc chlorid to assist in removing proteins and coloring matter (Folin. *J. Biol. Chem.* 1912, **11**: 529). Dilute to the mark with 95 per cent ethyl alcohol and mix. Let stand for a couple of hours, until the precipitate separates out. Filter through dry, folded filter. Take 75 cc. of the filtrate, transfer to a double-necked distillation flask and remove ammonia according to Van Slyke process; that is, add drop by drop to the filtrate enough 25 per cent sodium hydrate to make the filtrate alkaline to phenolphthalein. Again concentrate under diminished pressure to remove both ammonia and alcohol until the volume is 10-20 cc. Then add to the flask 50 per cent acetic acid until the solution is acid. Also add about 50 cc. of water and continue the concentration. Evaporate to 10-15 cc., and very cautiously transfer with water to a 50 cc. volumetric flask. Dilute to the mark and mix.

Take 25 cc. of the solution. Transfer to a 50 cc. volumetric flask. Make neutral or slightly alkaline to phenolphthalein. Dissolve any insoluble substances with the aid of tenth-normal sodium hydroxid, using not more than 5-6 cc. Keep the flask stoppered to avoid the presence of carbon dioxid. Add 20 cc. of the "buffer" solution and a little of the freshly made cupric hydroxid. Shake the mixture vigorously for about a minute. If there is an excess of the cupric hydrate, after bringing it to room temperature, dilute to the mark, and shake for 2-3 minutes. Filter through dry filters (S. and S. No. 590 11 cm.). The filtrate is supposed to contain all the soluble complexes and the insoluble residue on the filter the insoluble complexes and the excess of copper hydroxid.

An aliquot portion (25 cc.) of the filtrate is then taken and after acidification with 1-3 cc. of glacial acetic acid, 5 cc. of the potassium-iodid-starch solution are added and the solution titrated with four-thousandths-normal thiosulphate solution. Every cubic centimeter of thiosulphate solution is equivalent to 0.0003184 gram of cupric oxid or 0.0001120 gram of amino acid nitrogen or 1 cc. of one-thousandth-normal thiosulphate solution is equivalent to 0.0000280 gram of amino acid nitrogen.

(*b*) *Beef extracts.*—Take the 100 cc. of the alcoholic solution prepared by the Van Slyke method, transfer it to a 110 cc. volumetric flask and add a few drops of the alcoholic zinc chlorid solution. Dilute to the mark with 95 per cent ethyl alcohol and mix. Continue exactly as outlined for meats by this method.

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[1] Reprints by Kober and Sugiura accompanied instructions to collaborators.

REPORT ON FEEDS AND FEEDING STUFFS.

By G. L. Bidwell (Bureau of Chemistry, Washington, D. C.), *Referee.*

The subject of the nitrogen factor has been before the association for several years. The factor 6.25 has been used for nearly 70 years and, when recent additions to the knowledge of the proteins are considered, is it not proper to ask if that value is not antiquated, and if it is not time to change to one which will give results nearer the truth? When substances containing a few well-known proteins are considered, such as dairy products or wheat flours, there can be no argument but that the change should be made from 6.25 to a factor that is known to be nearer correct. But in the case of feeding stuffs, especially from a control or inspection standpoint, there is a different set of conditions. In the first place, there are about 100 different products that enter into the composition of stock feeds. Ten or fifteen different products are derived from corn alone. From wheat we get bran, middlings, red dog, ship stuff and wheat screenings. Because wheat has a factor of 5.7, it does not follow that bran, middlings, and red dog, have the same factor nor is it likely that bran has the same factor as red dog. As for screenings, how is the factor to be determined? If it should be determined, would there ever be another sample of screenings which would have the same factor?

Now, consider the labor involved in determining the factor to apply to any one substance. This is not a problem to be solved by some assistant in a few weeks. It would be necessary to determine the amount of each protein in that substance, then to prepare it in a pure condition so that the per cent of nitrogen might be determined therein. This is a problem for the specialist who has available almost unlimited time and money. But if there were factors of all the ingredients of feeds could they be used? About 45 per cent of the feeds examined in the Bureau of Chemistry for the past two years were mixed feeds, and even when coarsely ground, the trained microscopist could not tell, except within very wide limits, the amounts of the various ingredients present. If different factors were used for the various feed stuffs, it would still be necessary to have a general factor for mixtures. If the factor for corn meal should be 6 and wheat by-products 5.7 and a mixture of 97 per cent middlings and 3 per cent corn meal were being analyzed, the general factor would still have to be used, whatever that might be. Although 5.7 would be the factor that would give nearest correct results, it would be impossible to tell the relative amounts of middlings and corn meal and therefore the only course left would be to use the general factor. Now, keeping in mind that we are considering this from the control and inspection standpoint, is it ever desired to know the actual amount of protein in a feed? It is known that the different proteins vary a great deal in composition, some having less than 15 per cent nitrogen and others having over 17 per cent. But is there anything to show that a pound of one of these is equal to a pound of the other in feeding value? It seems reasonable to suppose that the amount of nitrogen may be a better index of the value of the proteins of a feed than the actual weight of the protein itself. For nearly 70 years the per cent of nitrogen has been multiplied by 6.25 and the result called protein, but all this time it is the nitrogen percentages that have been compared. If another factor were adopted the same ratios would still be used when comparing the protein content of feeds; that is, the percentages of nitrogen would still be used even though they were multiplied by a factor. There are several hundred thousands of analyses of feeds on record and nearly all of them employ the factor 6.25. If the factor were changed the results of analyses would no longer be comparable with former analyses. It is too much to hope that all chemists would adopt the new factor. Experience has also shown that it would be impossible to persuade all chemists to indicate the factor they employed. It seems, therefore, that a change would cause endless confusion and give no compensating advantages. Of course it should be understood that these arguments do not apply to investigational work or work that is restricted to single well-known substances such as those mentioned in the first part of this report.

RECOMMENDATIONS.

It is recommended—

(1) That 6.25 be retained as the factor to change nitrogen to protein in all feeding stuffs control and inspection work.

(2) That the subject of improvements in the determination of fiber be further studied.

REPORT ON CRUDE FIBER.

By Charles K. Francis (Agricultural Experiment Station, Stillwater, Okla.), *Associate Referee.*

The work on crude fiber has been confined to determinations on one sample of cottonseed meal. The sample was sent to the collaborators without special instructions, other than to determine the crude fiber and report the details of the method used.

The well-known official method for determining crude fiber has been subjected to criticism by many chemists. The chief trouble seems to be with the filtering materials, linen, asbestos, or glass wool. These substances vary in their physical characteristics and necessarily in filtering efficiency. The determinations reported show a sufficient range of materials, and certainly the results suggest a need for further study. Except where otherwise indicated, the official method was used by the collaborating chemists.

RESULTS OF COÖPERATIVE WORK.

Crude fiber in cottonseed meal.

COLLABORATOR	CRUDE FIBER	REMARKS
	per cent	
J. M. Pickel, Raleigh, N. C.	6.94	Filtration, for acid digestion, upward suction through linen stretched over a funnel.
	6.72	
	6.05	
	6.69	
	6.21	
	6.62	
Average	6.54	
C. G. Remsburg, College Park, Md.	7.99	Muslin filter.
	7.88	
	8.07	
	8.15	
Average	8.02	
C. Cutler, Lafayette, Ind.	7.85	Dried at 100°C. Linen filter.
	8.09	
	7.76	
	8.15	
	7.91	
Average	7.95	
J. H. Roop, Lafayette, Ind.	8.33	Dried at 100°C. Linen filter.
	8.15	
	8.30	
Average	8.26	
C. E. Mangels, Columbia, Mo.	8.54	Muslin filter.
	8.86	
	8.56	
	8.51	
Average	8.62	
C. E. Mangels, Columbia, Mo.	8.59	Sweeney method.
	8.53	
Average	8.56	
A. S. Wells, Portland, Ore.	10.96	Silk filter.
	11.42	
	11.20	
Average	11.19	
C. Kennedy, St. Paul, Minn.	10.77	Sweeney method, Kennedy modification. Linen filter.
	10.35	
Average	10.56	
A. A. Jones, Stillwater, Okla.	9.50	Sweeney method, modified. Asbestos filter.
	9.54	
Average	9.52	
O. C. Smith, Stillwater, Okla.	10.55	Sweeney method, modified. Asbestos filter.
	10.83	
	10.25	
Average	10.54	
G. Merry, Stillwater, Okla.	9.76	Sweeney method, modified. Asbestos filter.
	9.14	
	9.55	
Average	9.48	
J. W. Chewning, College Station, Texas	8.76	Asbestos filter.
G. W. Roark, College Station, Texas	8.99	Asbestos filter.
	8.67	
Average	8.83	
General average	9.07	

RECOMMENDATIONS.

It is recommended—

(1) That the Sweeney method, and some of its modifications, be studied.

(2) That asbestos be specified as the filtering material.

(3) That the official method be compared, with linen and asbestos filters specified.

THE DETERMINATION OF CRUDE FIBER.

By G. L. Bidwell and G. P. Walton (Bureau of Chemistry, Washington, D. C.).

DISCUSSION.

The official method for the determination of crude fiber is considered far from satisfactory by many feed analysts. This is apparently due to difficulties in manipulation and to the time necessary to complete the determination. Several modifications of the method have been proposed with the view of eliminating various objectionable features or of shortening the time required. An investigation of the official method and one of the more promising of the proposed modifications has been undertaken.

For this purpose 4 representative feeding stuffs, having a fairly wide range in crude fiber, were selected.

Description of samples used in this investigation.

SAMPLE	CRUDE FIBER
	per cent
Oats (containing oat hulls)	11.30
Corn (Argentine)	1.80
Cottonseed meal	12.33
Roughage (a composite of several forage plants)	26.57

[1] Average of 8 separate determinations by the official method.

A large sample of each was carefully prepared by grinding, sifting and thoroughly mixing.

Each sample was extracted with ether before the crude fiber determination was made.

Kennedy has proposed a modification of the Sweeney method, endeavoring to eliminate the acid filtration which is considered one of the principal drawbacks to the official method. Her method is as follows:

> Boil fat-free material for 30 minutes under a reflux condenser with 200 cc. of a 1.25 per cent solution of sulphuric acid, add 200 cc. of boiling 3.52 per cent solution of sodium hydroxid; boil 30 minutes longer, filter through linen, wash first with a little hot water, then thoroughly with a boiling 1.25 per cent solution of sulphuric

acid, and then with hot water, transfer to a Gooch crucible, finish washing with hot water, then with 95 per cent alcohol and finally with ether. Dry to constant weight at 110° C., weigh, incinerate, weigh, and the difference is crude fiber.

The following table gives the results obtained by this method:

Per cent of crude fiber.

SAMPLE	METHOD		
	Kennedy modification	Official	Excess of Kennedy over official
Oats	12.30	11.30	1.00
Corn	2.29	1.80	0.49
Cottonseed meal	13.56	12.33	1.23
Roughage	26.70	26.57	0.13

From the writers' experience the official method takes less time than the Kennedy modification. The slow final filtration, the time consumed by washing with the dilute sulphuric acid and transferring the residue from the linen to the crucible more than make up for the time consumed in the acid filtration in the official method. To throw light on the high result by the Kennedy method another set in duplicate was run varying from the official method only by the addition of a sufficient amount of sodium sulphate to both solutions to give the same concentration of this salt as that resulting from the neutralization of the sulphuric acid in the Kennedy method. Although this solution boiled at 102°C. its dissolving or disintegrating action was less than that of the regular reagent. The average percentage figures obtained were: oats 11.87; corn 2.18; cottonseed meal 13.51; and roughage 27.72.

A charge of cottonseed meal was boiled with the acid as in the official method. After filtration the acid filtrate was treated with 200 cc. of 3.52 per cent of sodium hydroxid solution. The precipitate formed was filtered and washed with boiling 1.25 per cent solution of sulphuric acid; 0.85 per cent, calculated on the original charge of cottonseed meal, remained undissolved.

Since crude fiber is not the name of a definite substance but only a convenient and somewhat descriptive term used to denote the result obtained by treating a feed by the official method, several determinations were made to show the effect of variations of those factors which define that method. When using 400 cc. for each of the reagents instead of 200 cc., the results were only a trifle less than by the official method. Changing the concentration of the reagent affected the results appreciably, as is shown in the following table:

Per cent of crude fiber obtained with solutions of varying percentage composition.

STRENGTH SOLUTION	OATS	CORN	COTTONSEED MEAL	ROUGHAGE
0.78	12.23	1.97	13.44	28.38
1.25	11.30	1.80	12.33	26.57
2.00	10.52	1.77	11.01	24.73

The percentage of crude fiber obtained is inversely proportional to the concentration of the reagents.

To show the effect of variation in the time of boiling, sets were run with the regular acid and alkali solutions, for the following periods of time: 20 minutes; 45 minutes; 60 minutes; and 90 minutes. The results showed a continuous decrease in the percentage of crude fiber with no indication that a point might be reached beyond which further boiling would cease to cause a decrease in crude fiber.

The rôle performed by each of the reagents was next investigated. Sets were run in duplicate with acid alone for 20, 30 and 45 minutes.

Per cent of residue obtained.

TIME OF BOILING	OATS	CORN	COTTONSEED MEAL	ROUGHAGE
minutes				
20	23.00	9.97	26.74	48.94
30	20.22	9.00	24.15	46.56
45	18.50	8.26	22.63	44.64

Considering the total material dissolved in the official method as 100 per cent, the amount dissolved by acid in the oats is 89.94 per cent; in the corn 92.67 per cent; in the cottonseed meal 86.85 per cent; and in the roughage 72.78 per cent.

SUMMARY.

Inasmuch as crude fiber designates simply a numerical value obtained by following the official method which is strictly an empirical one, any deviation therefrom will give incorrect results. This is the strongest and all important objection to any material change in a method for official endorsement. The writers have found that by observing certain precautions, the results by the official method will check very closely and the difficulties and tediousness will be eliminated. In fact, they have been unable to make the determination by any of the proposed modifications in any less time than by the official method.

PRECAUTIONS.

(1) The solutions should be of proper strength and mixed thoroughly.

(2) The charge should be practically free from fat and about 1–2 grams of ignited asbestos added to render the filtration quicker.

(3) The boiling should be under a reflux condenser to prevent concentration of the reagent.

(4) The time of boiling and filtration should be uniform, as the lengthening of either tends to lower results. The linen should be selected carefully. It is possible to filter and wash a set of 10 determinations obtaining a clear filtrate in about 15 minutes. This is true of cottonseed meals as well as the more readily filtered materials.

(5) The washings should be made with very hot water.

(6) The wash water after the acid filtration should be allowed to drain so as to avoid diluting the alkali solutions.

If these precautions are observed in conjunction with the directions found in U. S. Bur. Chem. Bull. 107, Rev., it is believed that little fault will be found with the official method.

GOSSYPOL, A TOXIC SUBSTANCE IN THE COTTONSEED.

A Preliminary Note by W. A. Withers and Frank E. Carruth
(Agricultural Experiment Station, Raleigh, N. C.).

Gossypol occurs in the peculiar resin glands of the cottonseed. Oil was removed from ground up cottonseed kernels by petroleum ether, leaving gossypol undissolved. This was subsequently extracted by ethyl ether. The gossypol extract was dissolved in purified cottonseed oil and fed to rabbits by catheter and also by intraperitoneal injection. It was fatal in every case in a few hours. The extract was mixed with corn meal and molasses and fed to rabbits. It was uniformly fatal in 9 to 16 days.

A product precipitated from the "gossypol extract" by petroleum ether was also poisonous.

A crystalline product corresponding in properties to the phenolic substance gossypol, isolated by Marchlewski[1] from crude cottonseed oil was dissolved in purified cottonseed oil and administered to 2 rabbits intraperitoneally. It caused death within 6 hours.

It is difficult to extract gossypol completely as this material is held dye-like by the seed tissue and some of the resin glands are perhaps not penetrated by the solvent. Thoroughly extracted kernels were fed over long periods without fatal results.

The properties of gossypol may be used to explain the fact that boiling alcoholic alkali removes the toxicity of cottonseed meal,[2] and iron salts act as *antidotes* for cottonseed meal.[3] In alkaline solutions the substance *oxidizes* with a purple coloration which subsequently disappears. Ferrous sulphate forms an insoluble lake with gossypol.

The occurrence and properties of gossypol are being further studied and will form the basis of a later report.

[1] *J. prakt. Chem.*, 1899, **60**: 80.
[2] *Science*, 1912, **36**: 31.
[3] *J. Biol. Chem.*, 1913, **15**: 161.

REPORT ON FEED ADULTERATION.

By Carleton Cutler (Experiment Station, Lafayette, Ind.), *Associate Referee.*

The study of feed adulteration was added by the association at its last meeting to the subjects for coöperative investigation. The growing importance of this subject is due to the many new sources of feeding stuffs resulting from increased demand and higher prices received for concentrated feeds. The utilization of numerous and varied by-products affords many opportunities for adulteration.

Because of the small amount of other data available, this report is confined to feeding stuffs inspected in the laboratory of the associate referee. Methods of adulteration have been outlined and a brief résumé prepared of some of the adulterations with methods for their detection.

For the examination of feeding stuffs for adulteration the following apparatus is necessary: a set of sieves from 20 to 70 mesh inclusive, a hand lens, a compound microscope with low and high power lenses, and a set of instruments, including dissecting needles, scalpels, etc. Certain reagents are necessary, such as clearing reagents and stains for cellulose, woody fiber, fats, oils, starch, proteid matter, etc. It is also desirable to have apparatus for making permanent mounts of substances of known purity for comparisons.

TYPES OF ADULTERATION AND METHODS OF DETECTION.

(*1*) *Total substitution of one material for another.*—Corn feed meal is sometimes substituted for hominy feed. Upon sieving the sample, a preponderance of fine starchy material and an absence of corn germ indicates adulteration. A quantitative determination of crude fat and protein is necessary for confirmation. Thirty-two samples of corn feed meal analyzed in the laboratory of the associate referee carried 3.9 per cent fat and 8.6 per cent protein as against 7.7 per cent fat and 11.2 per cent protein as the average of 72 samples of hominy feed.

(*2*) *Use of a substance of an inferior quality.*—Linseed meal containing ground flaxseed screenings is sometimes found. Upon sieving and examination a large amount of finely ground weed seed coatings is found. The characteristic odor of this adulterant is generally sufficient for detection. A protein determination generally gives confirmation.

(*3*) *Intentional addition.*—Cocoa shells may be added to a compounded feed. The cocoa shells can be separated from the coarser portion of the sample after sieving and can be identified by the practiced eye with certainty under the hand lens. The shell, chocolate brown on the outer surface and a silver brown on the inner surface, is thin and brittle to the touch of the dissecting needle. For confirmation one should clear, mount, and examine with the compound microscope. Some of the most prominent characteristics are the longitudinally elongated polygonal cells underlying the cross cells of the endocarp, the spiral cells of the spongy parenchyma and the thickened stone cells. The elements must be familiar from the examination of the pure substance.

Peat dried at a high temperature may be added to mixtures containing tankage, molasses and the like. No satisfactory method has been discovered for detecting this adulterant.

(4) *Accidental addition.*—Often in milling the flour stuff is not thoroughly removed from the bran coating. The microscopist who is familiar with wheat by-products can generally detect this with the hand lens after sieving. For confirmation, a quantitative determination shows a low content in both fat and protein.

(5) *Omission.*—Often in a proprietary feed one of the most expensive ingredients is omitted, the manufacturer, no doubt, trusting that because of the complexity of the mixture it will not be missed. Feeds of this class may be rubbed gently in the palm of the hand before sieving. This does not destroy the character of the individual ingredients, but renders them more recognizable for the examination under the hand lens. Frequently in finely ground feeds it is necessary to make repeated mounts for examination with the compound microscope before the absence or presence of the ingredient can be confirmed.

(6) *Intentional abstraction and addition.*—A linseed meal may contain 15 per cent of pressed flaxseed screenings and 10 per cent of medium cottonseed meal and be sold for linseed meal, as the fat and protein content will approximate closely with that of pure linseed meal. The odor will indicate flaxseed screenings. If some of the adulterated sample is placed on the moistened finger and rubbed, the characteristic yellow of the cottonseed meal is noticed. After rubbing gently in the palm of the hand and sieving, one can easily detect weed seed coatings, fragments of cottonseed hulls and fine particles of meal by use of the hand lens, and by the use of a dissecting needle can pick out a considerable percentage of cottonseed product.

Other adulterations observed are as follows:

Finely ground corn cobs added to hominy feed; white corn meal to wheat middlings; ground and whole wheat screenings to wheat bran and middlings; corn screenings, containing considerable cob, to chop feeds; clover seed and wheat screenings to poultry feeds; oat clipping by-product to proprietary feeds; and cottonseed hulls to cottonseed meal.

By the use of sieves, the hand lens and a compound microscope, the microscopist is able to detect these adulterations with considerable accuracy. A quantitative determination of protein, fat and fiber acts as a guide in detecting adulteration. The chances of adulteration are so numerous that the microscopist should be continually on the alert for new adulterants, and methods for their detection. It is important that he should be thoroughly familiar with feeding stuffs, their process of manufacture and the by-products used in the compounding of feeds.

The economic importance of adulteration is worthy of consideration, both from the viewpoint of the manufacturer and the consumer.

RECOMMENDATIONS.

It is recommended—

(1) That samples be sent out for the quantitative determination of adulterants in amounts varying from 5–25 grams to determine the smallest amount necessary to give concordant results.

(2) That samples be sent out containing unknown adulterants for qualitative detection of the same.

(3) That methods for the detection of peat dried at high temperature in feeding stuffs be investigated.

(4) That the maximum percentage of foreign materials permissible in mill by-products be investigated.

No report was made by the referee on sugar.

REPORT ON TESTING CHEMICAL REAGENTS.

By J. B. Rather (College Station, Texas), *Referee.*[1]

The work for the past year dealt with the following chemicals:

1. Crude caustic soda.
   a. Determination of nitrogen.
   b. Methods for the determination of sodium hydroxid and sodium carbonate.
2. Molybdic acid.
   a. Determination of molybdenum trioxid.
   b. Determination of phosphoric acid.
3. Ethyl ether.
   a. Determination of solids non-volatile at 100°C.
4. Citric acid.
   a. Determination of impurities.

CRUDE CAUSTIC SODA.

Determination of Nitrogen.

Method (A).—This method is similar to the method of Krauch-Merck for the determination of nitrogen in caustic soda, using, however, a 40 gram sample, which is the approximate amount of solid caustic soda used in the ordinary nitrogen determination.

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[1] Presented by G. S. Fraps.

Method (*B*).—In this method the above described procedure was modified by distilling the ammonia into *distilled water containing no acid*, then redistilling the distillate into fifth-normal hydrochloric acid in the usual manner.

Method (*C*).—In this method the procedure, outlined in (*B*), was further modified by the use of a blank titration of fifth-normal hydrochloric acid, which was intended as a correction for error of standard solution, titration, etc.

The results of the single distillation process (*A*) were quite variable, ranging from 0.03 to 1.75 cc. of fifth-normal acid. This was probably due to traces of alkali carried over in the distillate. The results by the redistillation process (*B*), which was devised to correct for this defect of method (*A*), vary from 0.03–0.75 cc. of fifth-normal acid.

Determination of Sodium Hydroxid and Sodium Carbonate.

The direct titration method with phenolphthalein and methyl orange for the determination of sodium hydroxid and sodium carbonate in caustic soda was compared with the method of Tillmans and Heublein (*Z. Angew. Chem.*, 1911, **24**: 874), and it was found that the latter method gave better results.

MOLYBDIC ACID.

Determination of Molybdenum Trioxid.

The method used was the lead molybdate method, recommended by Krauch-Merck, as modified by the referee as follows: After solution of the molybdenum trioxid in ammoniacal water, it was filtered to remove iron and alumina; and further, the precipitate was washed by decantation a number of times before filtering through a Gooch. The results by different analysts agreed well except in a few instances.

Effect of varying some conditions of the Krauch-Merck method.—The possible effect of a number of factors in the determination of molybdenum trioxid in molybdic acid by the lead method were studied in order to find a possible reason for the variations sometimes occurring in the analyses. The results of the investigation showed: (1) that the quantity of lead acetate used does not greatly affect the results; (2) that the purity of the lead acetate does not affect the determination (The same results were obtained using a sample of "technical" lead acetate as when using a so-called "chemically pure" crystallized sample.); (3) that the addition of enough sulphates to cause an increase of 0.25 per cent in the supposed molybdenum trioxid content of the sample, caused an increase of 0.20 per cent in the results (Larger amounts of sulphates caused larger increases, but not in proportion to the amount of sulphates added. Further study will be required to determine the effect of the presence of sulphates.); (4) that the acidity of the solution may be varied within wide limits without affecting the accuracy of the method.

Determination of Phosphoric Acid.

The method for the determination of phosphoric acid in molybdic acid as outlined in last year's report was further tested.

The results showed that small amounts of phosphoric acid (1 mg. or less) can not be determined always when the solution is diluted to 75 cc. or more. At a volume of 50 cc., all of the added phosphoric acid was recovered.

ETHYL ETHER.

Determination of Solids Non-Volatile at 100°C.

The method consisted in evaporating to dryness 100 cc. in a tared dish on the steam-bath and drying to constant weight at 100°C. The solids obtained varied from 0.0006–0.0034 gram. This would affect the results of a fat determination, calculated on the basis of a 2 gram sample, from 0.02–0.17 per cent. The results indicate that if ether be used for fat determinations, it should be tested for non-volatile solids regardless of its supposed purity.

CITRIC ACID.

Determination of Impurities.

This work was devised to study the accuracy of the methods used last year for the determination of sulphuric acid and oxalic acid in commercial citric acid.

Sulphuric acid.—This was determined in the ordinary way by precipitation with barium chlorid.

Oxalic acid.—This was determined by neutralizing with ammonia and precipitating with calcium chlorid in a strongly acidified acetic acid solution. After standing a few hours the precipitate was filtered, washed and ignited in the usual manner. If the solution is not strongly acid, calcium citrate precipitates when the solution is heated or stands overnight. Any moderate amount of the citrate will be removed in the washing of the precipitate of calcium oxalate.

The results show that the method for the determination of sulphates is adequate for amounts varying between 0.01 and 3.00 per cent. The results in the oxalate determination were not quite satisfactory.

RECOMMENDATIONS.

It is recommended—

(1) That the double distillation method (*B* including *C*) for the determination of nitrogen in crude caustic soda, the modified lead method for the determination of MoO_3 in molybdic acid, and the method for the determination of solids in ethyl ether non-volatile at 100°C., as given in this report, be studied further with the view of incorporating them in the official methods for nitrogen, phosphoric acid, and crude fat or ether extract, respectively.

(2) That the direct titration method and the Tillmans and Heublein method for the determination of NaOH and Na_2CO_3 in crude caustic soda and the method for the determination of P_2O_5 in molybdic acid, as given in this report, be studied coöperatively next year, and that the effect of the presence of sulphates on the determination of MoO_3 in molybdic acid, together with the causes of the variations in the determination of MoO_3 in molybdic acid by the modified lead method, be studied further.

(3) That the study of the nature and amount of the impurities in commercial citric acid be discontinued for the present.

A paper "On the Colorimetric Determination of Small Quantities of Hydrocyanic Acid" by Arno Viehoever and C. O. Johns of the Bureau of Chemistry, was read by Mr. Johns. This information has since been published (*J. Am. Chem. Soc.*, 1915, **37**: 601).

C. H. Jones presented the following recommendations of the Executive Committee, which were approved:

(1) That the proceedings of this association be published in the form of a quarterly.

(2) That a one-half day session be given to section meetings to be arranged by the Executive Committee.

(3) That the matter of the revision of the Constitution and By-Laws relating to annual dues be left until the 1915 meeting.

REPORT OF COMMITTEE A ON RECOMMENDATIONS OF REFEREES.

By B. B. Ross, (Agricultural Experiment Station), *Chairman.*

(Phosphoric acid, nitrogen, potash, soils, inorganic plant constituents, insecticides and water.)

PHOSPHORIC ACID.

It is recommended—

(1) That further work be done on the methods for basic slag as reported this year.

Approved.

(2) That special attention be given to standardizing the alkali solution used in the volumetric method.

Approved.

(3) That the methods given in this year's report be tried out on as many samples as possible.

Approved.

(4) That the associate referee give attention to the question of whether neutral ammonium citrate, sodium citrate, or citric acid solution should be employed as a solvent in the determination of reverted phosphoric acid in fertilizers.

Approved.

NITROGEN.

It is recommended—

(1) That the zinc-ferrous-sulphate-soda method for nitrates be further studied during the coming year with a view of its final adoption as official in 1916 and that it be now adopted as provisional.

Adopted, final action as provisional; approved for final action as official in 1916.

(2) That the Jones and Street methods for the determination of organic nitrogen activity be further studied during the coming year with the special purpose in view of improving or modifying the manipulations in the conduct of each process, so as to increase the accuracy of the water-insoluble organic nitrogen determinations, and, in the case of the Jones method, to overcome the difficulties experienced by most analysts in the distillation with alkaline permanganate; and that they be now adopted as official.

Adopted, final action.

POTASH.

It is recommended—

(1) That further study of the perchlorate method be made with special reference to the method for washing the potassium perchlorate precipitate.

Approved.

(2) That coöperation be secured to test the necessity for the addition of hydrochloric acid to the potash extract, with special reference to the reason why the hydrochloric acid is added.

Approved.

(3) That denatured alcohol made up according to formula 1 (U. S. Internal Rev. Reg. No. 30, Rev., Aug. 22, 1911, p. 45. To 100 parts by volume of ethyl alcohol, not less than 180° proof, there shall be added 10 parts by volume of approved wood alcohol and one-half of one part by volume of approved benzine) with sufficient water added to make 80 per

cent alcohol by volume, be further tested for washing potassium chloroplatinate, with a view of its adoption officially in 1915.

Approved for final action as official in 1915.

SOILS.

It is recommended—

(1) That a further test of methods for the determination of soil carbonates be made, comparing the Marr procedure with methods which involve the use of dilute hydrochloric acid (1 to 15) and constant aspiration of air with and without heating.

Approved.

(2) That the wet combustion method with the mixture of chromic and sulphuric acids for the estimation of organic carbon be further compared with combustion of the soil in furnace.

Approved.

(3) That, at the suggestion of O. M. Shedd of Kentucky, a study be made by the association of a method for lime requirement of soils by H. B. Hutchinson and K. MacLennan (*Chem. News*, Aug. 7, 1914, p. 61).

Approved.

(4) That the methods for nitrates, nitrites, and ammonia, as adopted for waters, in conformity with the recommendations of Committee A in 1913, be now adopted as official methods for the determinations of these constituents in aqueous soil extracts.

Approved for final action as official in 1915.

(5) That the official process for the determination of carbon dioxid in soils be stricken from the methods of the association.

Approved.

(6) That the official method for alkali waters be provisionally adopted for alkali soils.

Approved for final action in 1915.

INORGANIC PLANT CONSTITUENTS.

No report or recommendations.

INSECTICIDES.

It is recommended—

(1) That Method I for total arsenious oxid in Paris green, as described in U. S. Bur. Chem. Bull. 107, Rev., pp. 25–26, be changed as described in this year's report, and that the method thus modified be studied further with a view of its adoption as an official method in 1915.

Approved.

(2) That Methods II and III for total arsenious oxid in Paris green (U. S. Bur. Chem. Bull. 107, Rev., pp. 26–27) be discarded.

Approved.

(3) That Methods II and III for total arsenic in Paris green, described in this year's report, be studied further with a view of their adoption as official in 1915.

Approved.

(4) That the official method for the determination of moisture in Paris green be extended to include determinations of moisture in Bordeaux mixture, Bordeaux and Paris green mixtures, and Bordeaux and lead arsenate mixtures, when these materials are in the form of a powder, and that Method (b) for the determination of moisture in Bordeaux mixture, Bordeaux and Paris green mixtures, and Bordeaux and lead arsenate mixtures, when in the form of pastes, as described in this year's report, be studied further with a view of its adoption at the proper time as an official method.

Approved.

(5) That the method for the determination of carbon dioxid in Bordeaux mixture, Bordeaux and Paris green mixtures, and Bordeaux and lead arsenate mixtures, as described in this year's report, be studied further with a view of its adoption as an official method in 1915.

Approved.

(6) That the electrolytic method for the determination of copper in Bordeaux mixture, as described in this year's report, be studied further with a view of its adoption as an official method in 1915.

Approved.

(7) That the thiosulphate titration method for the determination of copper in Bordeaux mixture, as described in this year's report, be studied further with a view of its adoption as an official method in 1915.

Approved.

(8) That the method for water-soluble arsenious oxid in Bordeaux and Paris green mixtures, as described in this year's report, be studied further with a view of its future adoption as an official method.

Approved.

(9) That the distillation method for the determination of total arsenic in Bordeaux and Paris green mixtures, as described in this year's report, be studied further with a view of its future adoption as an official method.

Approved.

(10) That the methods of C. M. Smith and C. C. Hedges for the determination of total arsenious oxid in Paris green, as described in this year's report, be studied further with a view of their future adoption as official methods for the determination of arsenious oxid in Bordeaux and Paris green mixtures.

Approved.

(11) That the electrolytic method for the determination of copper in Bordeaux and lead arsenate mixtures, as described in this year's report,

be studied further with reference to its applicability to the determination of copper in both Bordeaux and Paris green mixtures and Bordeaux and lead arsenate mixtures.

Approved.

(12) That the thiosulphate titration method for the determination of copper in Bordeaux and Paris green mixtures, as described in this year's report, be studied further.

Approved.

(13) That the method for water-soluble arsenic oxid in Bordeaux and lead arsenate mixtures, as described in this year's report, be studied further with a view of its future adoption as an official method.

Approved.

(14) That the method for the determination of lead oxid in Bordeaux and lead arsenate mixtures, as described in this year's report, be studied further.

Approved.

(15) That Procedure (b) of the silicotungstate method for the determination of nicotin, as described in this year's report, be studied further with a view of its adoption as official in 1915.

Approved.

(16) That further work on the Lloyd method for the determination of nicotin be discontinued.

Adopted.

(17) That the coöperative work on insecticides for the coming year comprise a study of the following:

(a) Methods for the determination of As^{iii} and As^{v} in the presence of each other and in the presence of one or more of the following: lead, copper, zinc, and calcium.

(b) A method other than an electrolytic one for the determination of copper, lead, and arsenic in a Bordeaux and lead arsenate mixture.

(c) Methods for the determination of the principal ingredients in zinc-arsenic compounds, alone and in combination with Bordeaux mixture.

Approved.

WATER.

It is recommended—

(1) That methods for the determination of strontium be studied further during the coming year.

Approved.

REPORT OF COMMITTEE B ON RECOMMENDATIONS OF REFEREES.

By P. F. Trowbridge (Agricultural Experiment Station, Columbia, Mo.), *Acting Chairman.*

(Dairy products, feeds and feeding stuffs, sugar, water in foods and feeding stuffs, organic and inorganic phosphorus in foods, feeding stuffs, and drugs, separation of nitrogenous substances, testing chemical reagents, tannin, medicinal plants and drugs.)

DAIRY PRODUCTS.

It is recommended—

(1) That the copper serum method, as given in this year's report, be adopted as an optional provisional method for the detection of added water.

Adopted, final action.

(2) That the sour serum method, as given in this year's report, be adopted as an optional provisional method for the detection of added water.

Approved for final action as provisional in 1915.

(3) That the ash of the sour serum method, as given in this year's report, be adopted as an optional provisional method for the detection of added water.

Approved for final action as provisional in 1915.

(4) That the ash of the acetic serum method, as given in this year's report, be adopted as an optional provisional method for the detection of added water.

Approved for final action as provisional in 1915.

(5) That in conjunction with the copper acetic or sour serum refraction method, the determination of the ash of the sour serum or of the acetic serum be made in all cases where the indices of refraction fall below the minimum limit so as to eliminate all possibility of abnormal milk from a sick cow.

Approved.

(6) That further study be made of the Harding-Parkin method for fat determination (*J. Ind. Eng. Chem.*, 1913, **5**: 131) in comparison with the present official and provisional methods.

Approved.

(7) That in U. S. Bur. Chem. Bull. 107, Rev., p. 122, after the paragraph on Cream, the following paragraph be inserted under the heading "Condensed Milk (Unsweetened)"; "Dilute 40 grams of the homogeneous material with 60 grams of distilled water, proceed as directed

under 'Milk', and correct the results for dilution"; and the word "Sweetened" be inserted before the word "Condensed" in the subsequent heading.

Adopted.

(8) That further study be given to enzyme reactions of milk.

Approved.

FEEDS AND FEEDING STUFFS.

It is recommended—

(1) That no change be made in the nitrogen factor for protein.

Approved.

(2) That there be a thorough investigation of the methods for crude fiber determination.

Approved.

(3) That samples be sent out for the quantitative determination of adulterants in amounts varying from 5–25 grams to determine the smallest amount necessary to give concordant results.

Approved.

(4) That samples be sent out containing unknown adulterants for qualitative detection of the same.

Approved.

(5) That methods for the detection of peat dried at high temperature in feeding stuffs be investigated.

Approved.

(6) That the maximum percentage of foreign materials permissible in mill by-products be investigated.

Approved.

SUGAR.

No report or recommendations.

WATER IN FOODS AND FEEDING STUFFS.

It is recommended—

(1) That the investigation of desiccating agents, with and without increase in temperature, be continued.

Approved.

(2) That moisture determination by the vacuum method over sulphuric acid (U. S. Bur. Chem. Bull. 122, p. 219) be made an optional official method.

Recommended in 1913 and unconsidered in 1914.

ORGANIC AND INORGANIC PHOSPHORUS IN FOODS, FEEDING STUFFS, AND DRUGS.

It is recommended—

(1) That the magnesia mixture method of E. B. Forbes and his associates for the determination of water-soluble inorganic phosphorus in animal substances, as described in this year's report, be adopted as an official method.

Approved for final action as official in 1915.

(2) That further effort be made on methods of separation of organic and inorganic phosphorus in vegetable substances.

Approved.

SEPARATION OF NITROGENOUS SUBSTANCES (MEAT PROTEINS).

It is recommended—

(1) That work on ammonia and amino nitrogen be continued.

Approved.

(2) That the Folin method for estimating creatin and creatinin in meat and beef extract, as given in the 1913 report, be made official.

Adopted, final action.

(3) That the proposed method for determining the coagulable protein in meats (U. S. Bur. Chem. Bull. 107, Rev., p. 108, 7 (d)), be made optional.

Adopted, final action.

SEPARATION OF NITROGENOUS SUBSTANCES (VEGETABLE PROTEINS).

No report or recommendations.

SEPARATION OF NITROGENOUS SUBSTANCES (MILK AND CHEESE).

It is recommended—

(1) That studies be made leading to the adoption of methods for the determination of the noncasein proteins and the products of protein decomposition in milk.

Approved.

TESTING CHEMICAL REAGENTS.

It is recommended—

(1) That the double distillation method (B including C) for the determination of nitrogen in crude caustic soda, the modified lead method for the determination of molybdenum trioxid in molybdic acid, and the method for the determination of solids in ethyl ether non-volatile at 100°C., as given in this year's report, be studied further with the view of incorporating them in the official methods for nitrogen, phosphoric acid, and crude fat or ether extract, respectively.

Approved.

(2) That the direct titration method and the Tillmans and Heublein method for the determination of sodium hydroxid and sodium carbonate in crude caustic soda and the method for the determination of P_2O_5 in molybdic acid, as given in this year's report, be studied coöperatively this coming year, and that the effect of the presence of sulphates on the determination of molybdenum trioxid in molybdic acid, together with the cause of the variations in the determination of molybdenum trioxid in molybdic acid by the modified lead method, be studied further.

Approved.

(3) That the study of the nature and amount of the impurities in commercial citric acid be discontinued for the present.

Left for the next referee to decide.

TANNIN.

No report or recommendations.

MEDICINAL PLANTS AND DRUGS.

It is recommended—

(1) That the work on medicated soft drinks, especially the methods for estimating phosphoric acid and glycerin, be continued.

Approved.

(2) That the method for the determination of caffein and acetanilid, as given in this year's report, be adopted as a provisional method.

Approved by the committee on recommendations of referees for final action as provisional in 1915.

(3) That the method for the determination of caffein and antipyrin, as given in this year's report, be adopted as a provisional method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as provisional in 1915.

(4) That the method for the determination of caffein and acetphenetidin (U. S. Bur. Chem. Bull. 162, pp. 193–194), as slightly modified in this year's report, be adopted as an official method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as official in 1915.

(5) That the method for the determination of acetanilid and acetphenetidin, as given in this year's report, be adopted as a provisional method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as provisional in 1915.

(6) That the method for the determination of acetanilid and sodium salicylate, as given in this year's report, be adopted as a provisional method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as provisional in 1915.

(7) That the method for the determination of acetanilid and quinin sulphate, as given in this year's report, be adopted as a provisional method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as provisional in 1915.

(8) That the method for the determination of acetphenetidin and salol, as given in this year's report, be adopted as a provisional method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as provisional in 1915.

(9) That the method for the determination of caffein, acetanilid, and sodium bicarbonate (U. S. Bur. Chem. Bull. 132, pp. 197–198), as slightly modified in this year's report, be adopted as an official method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as official in 1915.

(10) That the method for the determination of caffein, acetanilid, sodium bicarbonate, and sodium bromid, as given in this year's report, be adopted as a provisional method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as provisional in 1915.

(11) That the method for the determination of caffein, acetanilid, sodium bicarbonate, and sodium salicylate, as given in this year's report, be adopted as a provisional method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as provisional in 1915.

(12) That the method for the determination of caffein, acetanilid, sodium bicarbonate, and codein sulphate, as given in this year's report, be adopted as a provisional method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as provisional in 1915.

(13) That the method for the determination of caffein, acetanilid, sodium bicarbonate, and quinin sulphate, as given in this year's report, be adopted as a provisional method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as provisional in 1915.

(14) That the method for the determination of caffein, acetanilid, sodium bicarbonate, sodium salicylate, and codein sulphate as given in this year's report, be adopted as a provisional method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as provisional in 1915.

(15) That the method for the determination of caffein, acetanilid, and sodium salicylate, as given in this year's report, be adopted as a provisional method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as provisional in 1915.

(16) That the method for the determination of alcohol, caffein, acetanilid, and sodium salicylate (U. S. Bur. Chem. Bull. 152, pp. 236–239), as slightly modified in this year's report, be adopted as a provisional method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as provisional in 1915.

(17) That the method for the determination of caffein, acetanilid, and codein sulphate (U. S. Bur. Chem. Bull. 162, pp. 195–196), as slightly modified in this year's report, be adopted as a provisional method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as provisional in 1915.

(18) That the method for the determination of caffein, acetanilid, and antipyrin, as given in this year's report, be adopted as a provisional method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as provisional in 1915.

(19) That the method for the determination of acetanilid, sodium salicylate, and codein sulphate, as given in this year's report, be adopted as a provisional method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as provisional in 1915.

(20) That the method for the determination of antipyrin, acetphenetidin, and codein sulphate (U. S. Bur. Chem. Bull. 162, pp. 197–198), as slightly modified in this year's report, be adopted as a provisional method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as provisional in 1915.

(21) That the method for the determination of acetanilid, quinin sulphate, and morphin sulphate, as given in this year's report, be adopted as a provisional method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as provisional in 1915.

(22) That the method for the determination of caffein, acetanilid, quinin sulphate, and morphin sulphate (U. S. Bur. Chem. Bull. 162, pp. 200–201), as slightly modified in this year's report, be adopted as a provisional method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as provisional in 1915.

(23) That the method for the determination of acetphenetidin and heroin, as given in this year's report, be adopted as a provisional method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as provisional in 1915.

(24) That the method for the determination of caffein, acetphenetidin and codein sulphate (U. S. Bur. Chem. Bull. 152, pp. 239–240), as slightly modified in this year's report, be adopted as a provisional method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as provisional in 1915.

(25) That the method for the determination of antipyrin and acetanilid (U. S. Bur. Chem. Bull. 152, pp. 240–241), as slightly modified in this year's report, be adopted as a provisional method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as provisional in 1915.

(26) That the method for the detection of thujone in liqueurs, as given in this year's report, be adopted as a provisional method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as provisional in 1915.

(27) That the method for the determination of santonin in Levant wormseed, as given in this year's report, be adopted as a provisional method.

Approved by the committees on recommendations of referees and editing methods of analysis for final action as provisional in 1915.

REPORT OF COMMITTEE C ON RECOMMENDATIONS OF REFEREES.

By H. E. Barnard (State Board of Health, Indianapolis, Ind.), *Chairman*,[1]

(Food adulteration.)

COLORS.

It is recommended—

(1) That the investigation of methods for the separation and identification of the important natural coloring matters be continued.

Approved.

SACCHARINE PRODUCTS.

It is recommended—

(1) That the work on the detection of artificial invert sugar in honey be continued for another year, studying the tests which proved the most satisfactory in the hands of all the collaborators, namely, (1) the original Fiehe test allowing the color to develop for twenty-four hours; (2) Hartmann's modification of the Fiehe test, noting the color immediately after the addition of the reagent; (3) Bryan's modification of the Fiehe test, changing the word "vigorously" to "gently"; (4) Feder's anilin chlorid test; with a view of adopting these methods as provisional in 1915.

Approved.

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[1] Presented by H. E. Bishop.

FRUIT PRODUCTS.

It is recommended—

(1) That the proposed methods for the determination of malic and citric acids, as given in this year's report, be given further study.

Approved.

WINE.

It is recommended—

(1) That the proposed method for the determination of total tartaric acid, as given in the 1913 report, be studied further with regard to its applicability to red wines.

Approved.

(2) That the suggested use of Rochelle salt addition instead of tartaric acid, as provided in the Hartmann and Eoff method, be further studied.

Approved.

BEER.

It is recommended—

(1) That Method 3 for the determination of phosphoric acid in beer by the addition of calcium acetate, and subsequent ashing, as given in the 1913 report, be adopted as a provisional method in place of the direct volumetric determination with uranium acetate.

Adopted, final action.

DISTILLED LIQUORS.

It is recommended—

(1) That the Allen-Marquardt method for fusel oil determination (U. S. Bur. Chem. Bull. 107, Rev., p. 98 (b)), be modified as follows: In the fourth line after "is collected" add "Whenever aldehydes are present in excess of 15 parts per 100,000, add to the distillate 0.5 gram of metaphenylene diamin hydrochlorid, reflux for an hour, distil 100 cc., add 25 cc. of water and continue distillation until an additional 25 cc. is collected."

Approved for further study with collaboration.

(2) That the associate referee be directed to confer with chemists engaged in spirits analysis, to do such collaborative work as is necessary, and to submit at the next meeting a revised Allen-Marquardt method containing such changes in details and manipulation as experience justifies.

Approved.

VINEGAR.

It is recommended—

(1) That Methods 6, 11, 15, 16, and 17, as given in U. S. Bur. Chem. Bull. 152, pp. 126 and 127, be adopted provisionally.

Approved for final action as provisional in 1915.

(2) That Methods 10 and 20, as given in U. S. Bur. Chem. Bull. 152, pp. 126 and 127, be given further study following the suggestions in U. S. Bur. of Chem. Bull. 162, p. 81.

Approved.

FLAVORING EXTRACTS.

It is recommended—

(1) That the saponification method of Hortvet and West for wintergreen extract, as described in the *J. Ind. Eng. Chem.* 1909, **1**: pp. 84–95, and slightly modified in U. S. Bur. Chem. Bull. 152, p. 141, be adopted as a provisional method.

Approved for final action as provisional in 1915.

(2) That the method of Hortvet and West for anise and nutmeg extracts, as described in the *J. Ind. Eng. Chem.* 1909, **1**: pp. 84–95, be given further study.

Approved.

(3) That the slight modification of the Howard-Mitchell method for peppermint extract, spearmint extract, and other extracts, be given further study.

Approved.

SPICES.

It is recommended—

(1) That final action regarding the lactic and citric acid methods be withheld pending further study and collection of data.

Approved.

(2) That the methods for determining insoluble solids and sand, as applied to ketchup (U. S. Bur. Chem. Bull. 162, pp. 128–129), be adopted as provisional methods.

Approved for final action as provisional in 1915.

MEAT AND FISH.

It is recommended—

(1) That the recommendations of 1913 be continued.

Approved.

FATS AND OILS.

It is recommended—

(1) That the Bureau of Animal Industry method for the detection of phytosterol in fats (U. S. B. A. I. Cir. 212) be adopted as a provisional method.

Approved for final action as provisional in 1915.

(2) That the digitonin method, as described in this year's report, be adopted as a provisional method.

Approved for final action as provisional in 1915.

DAIRY PRODUCTS.

It is recommended—

(1) That the method proposed in 1911 (U. S. Bur. Chem. Bull. 152, p. 101; Cir. 90, p. 10) as applied to evaporated milk, sweetened condensed milk, thin cream, and ice cream, and recommended in 1913 for final adoption as provisional in 1914, be given further study.

Approved.

(2) That special attention be given to the Roese-Gottlieb method with a view of correcting its deficiencies, if such exist, particularly with reference to its use in sweetened and unsweetened condensed milk.

Approved.

(3) That the modification for rich cream (U. S. Bur. Chem. Circ. 108, p. 12) be given further study with a view of its final adoption as provisional in 1914.

Recommended in 1913 and unconsidered in 1914.

CEREAL PRODUCTS.

It is recommended—

(1) That the recommendations of 1913 be continued.

Approved.

VEGETABLES.

It is recommended—

(1) That the recommendation of 1913 be continued.

Approved.

COCOA AND COCOA PRODUCTS.

It is recommended—

(1) That the associate referee for the coming year make a study of the manufacture of milk chocolate with the view of finding out whether or not the casein is rendered insoluble in the reagents by different methods of manufacture.

Approved.

TEA AND COFFEE.

It is recommended—

(1) That the Fuller method for the determination of caffein in tea and coffee be studied further with a view of improving the method of extraction and filtration.

Approved.

(2) That the Görter method be studied further with a view of purifying the caffein with sodium carbonate solution for direct weighing.

Approved.

(3) That the combination method, using the Fuller and Görter methods, as proposed by Hanson in this year's report, be studied.

Approved.

(4) That the modified Stahlschmidt method be given another trial for the determination of thein in tea.

Approved.

PRESERVATIVES.

It is recommended—

(1) That the Fincke method for formic acid (*Biochem. Z.*, 1913, **51:** 253) be adopted as a provisional method.

Approved for final action as provisional in 1915.

(2) That the natural occurrences of formic acid in food products be further investigated.

Approved.

(3) That the Wegner method (*Z. anal. Chem.*, 1903, **42:** 427) be submitted to trial as a confirmatory test and that steps be taken to secure a reliable quantitative method for the determination of saccharin in foods.

Approved.

HEAVY METALS IN FOODS.

It is recommended—

(1) That the methods for the determination of lead in baking powder and baking powder materials be made the subject of further study, with the coöperation, if possible, of the associate referee on baking powder.

Approved.

(2) That further study be made of the Gutzeit determination for arsenic as carried on this year, especially as to the proper strength of acid to be used.

Approved.

(3) That a study of some modification of the Marsh method for the determination of arsenic be made.

Approved.

(4) That the gravimetric tin method and Baker's volumetric tin method be studied further.

Approved.

(5) That the volumetric tin method of Bloomberg and Lourie be studied further.

Approved.

(6) That methods for the determination of copper, zinc, nickel, and aluminium in food products be made the subject of study by the association as soon as possible.

Approved.

BAKING POWDER.

It is recommended—

(1) That a comparative study be made of the Seeker, Exner, and Remington method for the determination of lead.

Approved.

A motion, made by William Frear, that the Chairmen of Committees A, B and C meet to discuss the method of preliminary research and the conduct of the work of the association, was carried.

REPORT OF COMMITTEE ON RESOLUTIONS.

By William Frear (State College, Pa.), *Chairman.*

Resolved, That, by the death, on July 31, 1914, of Prof. Francis H. Storer, Emeritus Professor of Agricultural Chemistry of Bussey Institution, Harvard University, America has lost one of her pioneers in the application of chemistry to the art of agriculture, a teacher whose widely inclusive studies, instructive writings, and earnestness in his chosen field, have done much to inspire the later generation of American agricultural chemists with a realization of the value of their science as applied to agriculture, and to impress upon the minds of the farming public also its importance in the solution of farm problems.

REPORT OF THE AUDITING COMMITTEE.

By R. T. Davidson (Polytechnic Institute, Blacksburg, Va.), *Chairman.*

The following report by the secretary-treasurer was examined by the auditing committee and found to be correct:

REPORT OF THE SECRETARY-TREASURER FOR THE YEAR ENDING NOVEMBER 15, 1914.

Receipts:	
Balance November 15, 1913	$120.02
Dues for the year 1912 to 1913 received from 3 States, (North Dakota, Tennessee, and Vermont) after the report for that year was submitted	6.00
Dues for the year ending November 15, 1914, from 58 Federal and State organizations	116.00
Total receipts	$242.02
Expenditures:	
Telephone calls, Raleigh Hotel, 1913 meeting	$0.65
Tips, Raleigh Hotel, 1913 meeting	3.00
January 2, 1914. Stamps	2.00
January 5, 1914. 500 Circulars, 300 one-cent envelopes, plain	12.85
January 14, 1914. 1 rubber stamp	0.70
June 15, 1914. 300 two-cent envelopes	6.47
June 29, 1914. 350 letter circulars	3.75
July 21, 1914. 500 stamped envelopes	11.00
July 30, 1914. 800 announcements	16.75
Expressage on badges for 1914 meeting	0.42
300 badges from Bastian Brothers Co	23.00
Total expenditures	$80.59
Balance November 16, 1914	$161.43

Respectfully submitted,
(Signed) C. L. Alsberg,
Secretary-Treasurer.

Examined and found correct:
(Signed) R. J. Davidson, *Chairman, Auditing Committee.*

H. C. Lythgoe made a motion, which was carried, that the constitution be so amended that municipal chemists be eligible to membership in the association, and that the matter be referred to the proper committee.

REPORT OF COMMITTEE ON NOMINATIONS.

By W. A. Withers (College of Agriculture, Raleigh, N. C.), *Chairman.*

The committee submitted the following nominations for officers for the year ending November 1915: President, C. H. Jones, of Vermont; Vice-President, R. N. Brackett, of South Carolina; Secretary-Treasurer, C. L. Alsberg, of Washington, D. C.; additional members of the executive committee, Julius Hortvet of Minnesota, and E. F. Ladd of North Dakota.

The secretary was instructed to cast the unanimous ballot of the association for these officers.

No report was made by the referee on tannin.

The subject of tannin was dropped from further study by the association.

The association adjourned at 12.30 P.M. to reassemble at 1.30 P.M.

THIRD DAY.

WEDNESDAY—AFTERNOON SESSION.

REPORT ON MEDICINAL PLANTS AND DRUGS.

By W. O. Emery (Bureau of Chemistry, Washington, D. C.), *Referee.*

RECOMMENDATIONS.

It is recommended—

(1) That the work on medicated soft drinks, especially the methods for estimating phosphoric acid and glycerin, be continued.

(2) That the following method for the estimation of caffein and acetanilid in mixtures, be adopted as official:

CAFFEIN AND ACETANILID.

(W. O. EMERY METHOD.)

Preliminary Treatment.

I. If the sample is already in powder form, reduce to the finest possible condition and uniformity by rubbing in a mortar, then keep in a tightly corked tube or flask until required for analysis. Powders in paper, cachet or capsule containers are frequently of such fineness as to require little further trituration except to produce a more uniform product, in which event the above treatment should be performed carefully prior to the weighing of the sample. On a small tared filter (5.5 cm.) weigh out 0.3–0.5 gram of the product thus prepared, or, if preferred, an amount equal to, or a multiple of, the average unit dose (previously ascertained by weighing collectively 20 or more such doses), wash with successive small portions of residue-free chloroform, 30-50 cc., sufficient at least to insure complete extraction. Collect the solvent in a 200 cc. Erlenmeyer, then distil by gentle heat to about 10 cc., using in this and similar operations, if available, a small spray trap, substantially like that shown in U. S. Bur. Chem. Bull. 152, p. 239. Add 10 cc. of dilute sulphuric acid (1 to 10), remove to a steam-bath and proceed as directed under caffein.

II. If the caffein is present in the citrated form, or the composition of the mixture is such as to preclude complete extraction by the filter method, weigh out on a metal scoop or watch glass the desired amount of the prepared sample, transfer to a separatory funnel of the Squibb type, add 50 cc. of chloroform and 20 cc. of water, shake vigorously and after clearing, draw off the lower layer through a small dry filter into a 200 cc. Erlenmeyer. In the case of coated tablets and pills, take one or more for each determination, first crushing them in a mortar before treatment in a separator. Repeat the extraction twice with two 50 cc. portions of chloroform, on the completion of which any caffein-acetanilid mixture observable about the apex of the delivery tube of the separator or edge of the filter and tip of the funnel should be recovered very carefully by judicious washing with chloroform, such washings being subsequently united with the main portion. Distil the combined chloroformic portions to about 10 cc., then proceed as directed under caffein.

III. If the product under examination be an aqueous-alcoholic solution, evaporate a measured quantity on the steam-bath (or take an aliquot of the residue from an alcohol determination) until most of the alcohol has been expelled, transfer to a separatory funnel by pouring and rinsing with a minimum of water so that the final volume does not greatly exceed 20 cc., then, in order to regenerate any acetanilid possibly hydrolyzed during evaporation, add a little solid sodium bicarbonate, 1 drop of acetic anhydrid and 50 cc. of chloroform, shake vigorously and, after clearing, draw off the solvent through a filter into a 200 cc. Erlenmeyer. If the preparation contains alkaloids, acidify with a few drops of dilute sulphuric acid immediately after the above prescribed acetylation and prior to treatment with chloroform, in order to insure retention of such basic material in aqueous solution. Repeat the extraction twice with two 50 cc. portions of chloroform and treat the solvent substantially as in the two preceding cases preliminary to hydrolysis as outlined under caffein.

A gross separation of the active ingredients from vehicular matter having been effected in accordance with one of the above procedures, the next step consists in the conversion of acetanilid into acetic acid and anilin sulphate, from which caffein is readily separable by means of chloroform, the anilin salt being under the conditions of the experiment insoluble in this medium. The acid hydrolysis of acetanilid leaves caffein intact.

Determination.

Caffein ($C_8H_{10}N_4O_2$), *melting point about 235°C.*—Digest on the steam-bath the aqueous acid mixture containing caffein and acetanilid until the contents of the flask are reduced to 5 cc., add 10 cc. of water and continue the digestion until the liquid is again reduced to 5 cc., cool, pour and rinse into a separatory funnel with a minimum of water, so that the final volume does not greatly exceed 20 cc. Add 50 cc. of chloroform, extract in the usual way and after clearing withdraw the lower layer through a small dry filter into a 200 cc. Erlenmeyer. Repeat the extraction with two 50 cc. portions of chloroform. On completion of the third and final extraction, recover carefully all traces of caffein observable about the tip of the delivery tube and the edge of the filter, then distil the combined solvent to about 10 cc., finally transfer the residual liquid, by pouring and washing with chloroform, to a tared beaker or crystallizing dish, and allow the solution to evaporate spontaneously or by gentle heat and blast to apparent dryness. Cool and weigh until constant.

Acetanilid ($C_6H_5NHCOCH_3$), *melting point 113° C.*—Draw off into the Erlenmeyer the aqueous-acid solution of anilin sulphate remaining in the separator used in effecting hydrolysis, rinse several times with 5 cc. portions of water to insure complete removal of former contents, and then heat the liquid for 10 minutes on the steam-bath to expel all traces of chloroform. Wash the filter, used in the preceding operation to dry the chloroformic solution of caffein, once with 5 cc. of water, and add later to the main solution of anilin sulphate. Add 10 cc. of concentrated hydrochloric acid, then run in a standard solution of bromin (1 cc. of which is equivalent to about 5 or 10 mg. of acetanilid) until a faint yellow coloration persists. While adding this reagent, or after each small addition thereof, rotate the flask sufficiently to agglomerate the precipitated tribromoanilin, thus clarifying the supernatant liquid and rendering the state of titration more apparent to the operator. Multiply the number of cc. required to complete the precipitation by the value of 1 cc. in terms of acetanilid in order to ascertain the quantity of this substance originally present in the sample taken.

COMMENTS AND SUGGESTIONS BY THE REFEREE.

Tared filters for use in this and similar work may be conveniently prepared as follows: Fold, adjust dry in a short-stemmed funnel of about 1.5 inches in diameter, moisten and fit carefully so that the entire rim of filter closes snugly onto the glass. After drying, keep in a balance case or other convenient receptacle and when needed weigh on a small glass tripod, an appliance easily constructed in the laboratory.

It is self-evident that all manipulations with tared filters at the balance should be performed under comparable conditions. If, for example, the first weight is taken when the hygrometer indicates a humidity of 50 per cent, the second weighing should clearly be made only when the moisture content of the air is approximately the same. This is indispensable when it is desired to estimate the quantity of chloroform-insoluble residue (as sodium bicarbonate in acetanilid compound) or excipient (starch, sugar, talc, etc.) employed, as in tablets containing but one active ingredient. Experience has shown, however, that a variation in humidity up to 5 per cent between the 2 weighings would not be productive of material error.

It not infrequently happens that certain brands of compressed tablets, when treated on the filter according to Procedure I do not yield to chloroform all the drug actually present in the mass. Such failure in apparently simple combinations to give up all their active principles to a suitable solvent is unquestionably due to the nature of excipient employed in the process of granulation. Starch paste, sirup, dextrin, gum, etc., as ordinarily applied, may easily and doubtless do dissolve and on drying hold or occlude appreciable quantities of any substance possessing a fair degree of solubility in aqueous media. In support of this assumption may be mentioned the fact that, in the case of certain caffein-acetanilid tablets, the chloroform-insoluble residue even after exhaustive treatment with the solvent still showed traces of the active principles in question, while in tablets containing the more insoluble acetphenetidin, no such resistance to extraction was observed. One should therefore not be surprised, in applying the first or filter treatment to caffein-acetanilid mixtures, to discover appreciable amounts of these drugs persisting in the chloroform-insoluble residue. While the quantity thus occluded is usually so small as to be almost negligible, the careful analyst will undoubtedly desire to check the accuracy of the first procedure by applying the second also, involving the use of a separatory funnel. In operating, therefore, according to Procedure I it is wise to apply the isocyanid test to the chloroform-insoluble residue, in order to prove the absence of even traces of acetanilid, which may be present in the occluded state in the powdered mass. As a means of control or in very exact work, it is recommended that the sample be subjected to both Procedures I and II.

Before making an extraction in a separatory funnel, the valve should be "locked" with a drop of water in order to reduce the tendency to loss from capillarity. All chloroform used in drug analysis should be carefully redistilled, unless it is positively known to be a residue-free product. It should also be neutral in reaction. All cork stoppers employed in connection with the distillation of chloroform should have been previously digested with this solvent a sufficient length of time to remove all extractives, which would otherwise contaminate the substances in course of estimation, and thus vitiate the results.

In the operation of withdrawing chloroform from a separatory funnel on completion of an extraction, it will frequently be found advantageous to free the solvent as much as possible from mechanically suspended water, which would otherwise be carried along and in the end contaminate, by virtue of dissolved salts, the substance it is desired to estimate. The chloroform is accordingly made to pass first, through a small, dry pledget of chloroform-washed cotton, pushed up into the delivery tube of separator, and second, a small (5.5 cm.) dry filter prior to reception in an Erlenmeyer from which distillation is effected. After each withdrawal of chloroform, the cotton is removed to the separator by means of a wire, and, on completion of the following extraction, a fresh pledget substituted in its place. The filter is allowed to dry completely between extractions.

Preparations containing, in addition to caffein and acetanilid, powdered cinnamon, celery seed, ginger or other vegetable products, yield to chloroform certain oils, fats, waxes, resins, pigments and other extractives, which in due course appear either in suspension or solution after the caffein-acetanilid mixture has been subjected to digestion, and finally also contaminate the caffein as first isolated. Any suspended impurities may be eliminated readily by passing the aqueous-acid liquid through a small moistened filter tightly packed in a funnel, prior to extraction with chloroform. Should the caffein, as first recovered and weighed, be deeply colored or apparently contaminated with foreign matter, it may be purified in the following way: Dissolve in very dilute sulphuric acid, pass, if necessary, through a moistened filter to eliminate suspended impurities, add to the clear solution 15–20 cc. of Wagner's reagent, sufficient at least to render the supernatant liquid distinctly claret in color, stir, then allow to stand an hour. Filter and wash the peroxid with a few cc. of iodin solution, transfer both filter and precipitate to a separatory funnel, using for this purpose not more than 20 cc. of water, decolorize with a crystal of thiosulphate, then extract with three 50 cc. portions of chloroform and proceed as directed under caffein.

To prepare a standard solution of bromin, add bromin in slight excess to a concentrated aqueous solution of caustic potash (50 grams), dilute with water to dissolve any separated salts, boil to expel excess of bromin, and finally dilute to 1 liter. Standardize this solution with weighed amounts of pure anilin, or better with recrystallized and dried acetanilid, and finally adjust by proper dilution until 1 cc. is equivalent to 5 or 10 mg. of acetanilid.

Caffein and acetanilid are the two principal ingredients of the preparation known as "acetanilid compound", a further constituent being sodium bicarbonate. The last named substance appears as the chloroform-insoluble residue and may be accurately determined by titrating such residue, or one obtained by igniting a portion of the original mixture, with standard acid in the presence of congo red as indicator. The bicarbonate may likewise be determined by igniting the original sample, or the chloroform-insoluble residue, with sulphuric acid and weighing the resulting sodium sulphate.

Should the "acetanilid compound" be combined with sodium bromid, the latter substance, in the absence of other halids, is easily estimated volumetrically by means of standard silver nitrate in combination with potassium sulphocyanid and ferric ammonium sulphate.

(3) That the following method for the estimation of caffein and acetphenetidin (phenacetin) in mixtures be adopted as provisional:

CAFFEIN AND ACETPHENETIDIN.

(W. O. EMERY METHOD.)

Preliminary Treatment.

With preparations containing acetphenetidin (phenacetin) instead of acetanilid, but otherwise identical, a gross separation of the caffein-acetphenetidin mixture is effected by practically the same preliminary treatment as that accorded caffein-acetanilid. Only in the matter of subsequent hydrolysis and regeneration of acetphenetidin is it necessary to observe the most careful circumspection as regards minor details involved in the various operations.

Caffein.—After distilling the chloroformic solution of caffein-acetphenetidin to about 10 cc., add 10 cc. of dilute sulphuric acid (1 to 10), then remove to the steam-bath and digest until the liquid is reduced to about 5 cc. Dilute with 10 cc. of water and continue the digestion until the volume is again reduced to 5 cc., then add a second and final 10 cc. of water and continue heating until the residual liquid amounts to 8–10 cc. If, during the process of digestion, particles of acetphenetidin persist on the sides of the flask, add, from time to time, a few drops of chloroform in order to bring such particles more completely within the radius of hydrolytic action. Cool and transfer to a separatory funnel by pouring and rinsing with water, until the final volume of liquid does not greatly exceed 20 cc. Extract, by means of vigorous shaking, with three 50 cc. portions of chloroform, manipulating the solvent and caffein substantially as under caffein-acetanilid treatment.

Acetphenetidin ($C_2H_5OC_6H_4NHCOCH_3$), *melting point 135°C.*—Wash the filter used to dry the chloroform in the preceding operation once with 5 cc. of water, receiving the latter in the separatory funnel containing the aqueous-acid solution of phenetidin sulphate. Treat with successive small portions of solid sodium bicarbonate until, after complete neutralization of free acid, an excess of the former persists at the bottom of the mixture. Then add 50 cc. of chloroform and, for every 100 mg. of acetphenetidin known or believed to have been present, 5 drops of acetic anhydrid; shake vigorously for some time, allow to clear, then withdraw the chloroform into a second separator containing 5 cc. of water. Shake this mixture and, after clearing, pass the solvent through a small dry filter into a 200 cc. Erlenmeyer. Distil over about 40 cc. of the chloroform, make up to 50 cc. with fresh solvent and extract again. Withdraw to the second separator, wash and distil as before, about 50 cc., which are applied on the third and final extraction. Then distil the chloroform to about 10 cc., transfer the residue to a 50 cc. tared crystallizing dish or beaker, by pouring and rinsing with sufficient fresh solvent, evaporate on the steam-bath to apparent dryness, finally removing any considerable excess of acetic anhydrid by repeated additions of 1 cc. of fresh chloroform, to which has been added a drop of alcohol. The regenerated acetphenetidin should finally appear as a whitish, crystalline mass with a faint acetous odor. The latter will completely disappear on standing some hours in the open, or in a vacuum desiccator over lime. Weigh from time to time until the final weight differs from the preceding not more than 0.5 mg.

COMMENTS AND SUGGESTIONS BY THE REFEREE.

Acetphenetidin, owing to its greater insolubility in aqueous-acid media, is correspondingly more difficult of hydrolysis than acetanilid. In order therefore to insure complete conversion to phenetidin sulphate, special attention should be directed to the end that any crystalline deposits (acetphenetidin), which may appear in the beginning of the operation on the sides of the flask just above the liquid, should be forced back into the hot liquid by an occasional gentle rotation of the flask or, better, by the occasional addition of a few drops of chloroform. It is self-evident that, if hydrolysis is incomplete, subsequent extraction with chloroform will remove not only caffein but also any acetphenetidin that may have escaped hydrolytic action, and thus vitiate the results for caffein. Great care too should be given the degree of evaporation. Should the aqueous-acid solution and suspension of caffein-acetphenetidin be concentrated far beyond the limits indicated in the method, more or less phenetidin sulphonate is likely to be formed, which later resists acetylization and conversion to acetphenetidin.

(4) That the method for the estimation of caffein and antipyrin in mixtures,[1] as given in this year's report, be adopted as provisional:

CAFFEIN AND ANTIPYRIN.

(W. O. EMERY AND S. PALKIN METHOD.)

Antipyrin ($C_{11}H_{12}N_2O$), *melting point 113°C.*—Transfer about 0.25 gram of the caffein-antipyrin mixture to a 150 cc. separatory funnel by means of two 5 cc. portions of alcohol-free chloroform followed by 10 cc. of water, add 1 gram of sodium bicarbonate and 10–15 cc. of fifth-normal iodin (or double the quantity of tenth-normal iodin), this reagent being applied in successive small portions and the mixture shaken vigorously 15–20 seconds after each addition. When all the iodin has been thus added, a decided excess of this reagent should be apparent in the liquid after a final vigorous shaking of 1 minute, in which event all the antipyrin will have been converted into the moniodo derivative. If, however, all the iodin previously applied should appear to have been expended, a little more must be added to insure an excess, and the mixture again shaken. Now discharge the uncombined iodin by means of a small crystal of sodium thiosulphate, add 15 cc. of redistilled U. S. P. chloroform, shaking vigorously for 1 minute. After clearing, wash the solvent in a second separator with 5 cc. of water, finally passing through a small dry filter into a tared 50 cc. beaker and evaporating to apparent dryness on the steam-bath, accelerating this operation with an air blast. Repeat the extraction with two (three, in case tenth-normal iodin is used) 25 cc. portions of chloroform, and wash, filter and evaporate each portion in rotation as in the first instance. Recover any crystalline product separating about the tips of the delivery tube and the funnel, and the edge of the filter, by judicious washing with chloroform. Dry the nearly colorless crystalline residue of caffein and iodoantipyrin for 30 minutes at 105°C., then cool and weigh. Designate this weight *A*.

Dissolve the composite residue in 5 cc. of glacial acetic acid, add 10 cc. of saturated aqueous sulphur dioxid solution, then transfer the resulting liquid by diluting, pouring and rinsing with hot water to a 400–500 cc. beaker until the final volume amounts to about 200 cc. Add aqueous silver nitrate solution (about 0.3 gram of silver nitrate), sufficient at least to precipitate all the halogen, follow with a few drops of nitric acid, then heat nearly to boiling, stirring the while in order to agglomerate the silver iodid. Add 15 cc. of concentrated nitric acid, cover the beaker with a watch glass and boil gently for 5 minutes. Filter by decantation through a tared Gooch, washing the precipitate once with a little alcohol, then with two 100 cc. portions of boiling water, and finally transfer the iodid completely to the crucible. Wash several times with hot water and again with alcohol to remove traces of organic matter, prior to drying for 30 minutes in the air-bath at 110°C. Cool and weigh. The weight of silver iodid multiplied by the factor 0.8012 yields the quantity of antipyrin present in the mixture.

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[1] *J. Ind. Eng. Chem.*, 1915, **7**: 519.

Caffein.—Ascertain the quantity of caffein by subtracting the product obtained by multiplying the weight of silver iodid by the factor 1.3374 from the weight of residue *A*.

COMMENTS AND SUGGESTIONS BY THE REFEREE.

In practice, the preliminary or gross separation of the caffein-antipyrin mixture from the usual excipients of tablet and pill combinations is most conveniently effected by extraction of the finely powdered mass with chloroform on a tared filter, this solvent, together with its attendant alcohol, being subsequently expelled completely, first by gentle distillation and finally by evaporation on the steam-bath, prior to proceeding with the separation proper. Likewise in the case of alcoholic preparations containing caffein and antipyrin as essential ingredients, the recovery of these two substances is best made after evaporation of the alcohol on the steam-bath. The residual menstruum is thereupon exhausted with chloroform in the usual way, the resulting solution of caffein and antipyrin being treated precisely like the one obtained from tablet or pill combinations.

The use of alcohol-free chloroform in connection with the halogenation of antipyrin is necessary in order to preclude the possible formation of iodoform, the presence of which in the composite residue *A* would naturally vitiate the analytical findings.

The application of iodin in small portions appears to favor the production of a purer iodoantipyrin than when this reagent is added all at one time, as evidenced by the color of the caffein-antipyrin residue. In order to free the latter from all contaminating volatile products under the conditions outlined in the method, particular attention should be given to actual working conditions of the drying oven,[1] since incomplete or improper desiccation must lead necessarily to widely divergent caffein values, while heating at temperatures materially higher than 105°C. is found to exert an unfavorable influence on the recovery of both caffein and antipyrin.

The color changes following the addition of strong nitric acid are quite characteristic, passing from colorless through deep red to pale yellow in the course of 5 minutes' boiling. In view of this somewhat radical treatment to which the caffein is likewise subjected, any direct determination of this substance becomes impractical. The quantity of caffein is ascertained indirectly therefore with a reasonable degree of accuracy by subtracting the weight of iodoantipyrin (calculated) from that of the composite residue *A*.

In the analysis of a mixture containing caffein, antipyrin, acetanilid and sodium salicylate, the following essential steps are suggested in effecting a separation:

(1) Extraction of caffein, acetanilid and antipyrin from the aqueous soda solution.

(2) Hydrolytic treatment of the three substances eliminated with sulphuric acid preliminary to the isolation of caffein and antipyrin, which are thereupon subjected to the procedure just described.

(5) That the following method for the estimation of acetanilid and acetphenetidin (phenacetin) in admixture,[2] be adopted as provisional:

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[1] *J. Ind. Eng. Chem.*, 1914, **6**: 585.

[2] Ibid., 665.

ACETANILID AND ACETPHENETIDIN.

(W. O. EMERY METHOD.)

Acetphenetidin.—Introduce 0.2 gram of the acetphenetidin-acetanilid mixture into a small (50 cc.) lipped Erlenmeyer flask, add 2 cc. of glacial acetic acid, heat gently over wire gauze to complete solution, then dilute with 40 cc. of water, previously warmed to 70°C. Transfer the clear acetous liquid to a glass stoppered, graduated 100 cc. flask, by pouring and careful washing of the flask with two 10 cc. portions of warm (40°C.) water into which have been run previously from a burette 25 cc. of standard iodin of a strength slightly above N/5 and warmed to 40°C. Rotate the resulting menstruum to uniformity, closing the flask meanwhile, then add 3 cc. of concentrated hydrochloric acid, close the flask anew and continue the rotation until copious crystallization is apparent, then set aside to cool. If the ratio of acetphenetidin to acetanilid is equal to or greater than 1, the formation of crystalline scales will be almost immediate on the addition of acid. As the proportion of acetanilid increases, however, the periodid is not only more inclined to maintain the liquid state, with the result that crystallization becomes proportionately slower, but its separation also from the menstruum itself is in a measure apparently retarded. In such cases, gentle agitation of the liquid or rotation of the flask in water warmed to 40°C. or less tends to promote the formation of crystals. When the contents of the flask have assumed the room temperature, fill with water to within 2–3 cc. of the mark, rotate to uniformity and allow to stand overnight. Fill to the mark with water, mix thoroughly, then after standing for 30 minutes withdraw a 50 cc. aliquot of the clear liquid by passing through a small (5.5 cm.) dry, closely fitted filter into a graduated 50 cc. flask, rejecting, however, about 15 cc. of the first runnings, the latter being received in any convenient container for eventual later use, along with the additional filtrate, for the recovery of acetanilid. Transfer the 50 cc. aliquot to a 200 cc. Erlenmeyer flask by pouring and washing and titrate with tenth-normal sodium thiosulphate.

If reference is had to the composition of the insoluble addition product, constituting the basis for the foregoing separation,

$$(C_2H_5O.C_6H_4NH.COCH_3)_2HI.I_4,$$

it will be noted that, for every molecule of acetphenetidin involved, 2 atoms of iodin are required, hence from a titrimetric standpoint 1 atom of iodin is equivalent to one-half molecule of acetphenetidin. If, therefore, the quantity of iodin expended in the formation of insoluble periodid is ascertained as the result of such titration, the quantity of acetphenetidin thereby involved is readily calculated from the expression,

$$\text{acetphenetidin} = I(0.008890 \times N),$$

in which 0.008890 represents the quantity of acetphenetidin in 1 cc. of a tenth-normal solution of this substance, N the normality of a standard thiosulphate employed, while I represents the number of cc. of such thiosulphate corresponding to the iodin entering into combination with phenacetin isolated as periodid.

The gravimetric determination of acetphenetidin may, if desired, be effected substantially as follows: Filter off the periodid, collect it on the filter and wash with 10–15 cc. of standard iodin solution, preferably by suction, then transfer, together with filter (likewise any particles of precipitate eventually remaining in the graduated flask), to a separatory funnel, using not over 50 cc. of water. After discharging both free and added iodin with a few small crystals of sodium sulphite, extract the liquid with three 50 cc. portions of chloroform, subsequently washing each portion in a second separatory funnel with 5 cc. of water. After washing and clearing, withdraw the solvent through a small (5.5 cm.) dry filter into a 200 cc. Erlenmeyer flask, distil off most of the chloroform, transfer the residual 5–10 cc. to a small tared beaker or crystallizing dish by pouring and washing with fresh solvent. Evaporate to dryness on the steam-bath, cool, and weigh.

Acetanilid.—Should the combined weight of the acetphenetidin-acetanilid mixture be known, that of the latter constituent can be determined by difference or, if necessary, estimated directly from a second aliquot of the filtrate from the acetphenetidin periodid.

To this end, transfer 25–30 cc. of the clear liquid to a separatory funnel by means of a pipette, decolorize with sufficient solid sodium sulphite, and solid sodium bicarbonate in slight excess, follow with 1 or 2 drops of acetic anhydrid, then extract with three 60 cc. portions of chloroform, passing the solvent when cleared through a small, dry filter into a 200 cc. Erlenmeyer, from which the chloroform is distilled, by the aid of gentle heat, to about 20 cc. Then add 10 cc. of dilute sulphuric acid (1 to 10) and digest the product on the steam-bath until the aqueous residue has been reduced one half, add 20 cc. of water and continue the digestion for an hour, add a second 20 cc. portion of water and 10 cc. of concentrated hydrochloric acid, then titrate very slowly, drop by drop, with standard potassium bromid-bromate (1 cc. of which is equivalent to 5–10 mg. of acetanilid), until a faint yellow coloration persists. While adding this reagent, the flask should be rotated sufficiently to agglomerate the precipitated tribromoanilin and thus clarify the supernatant liquid. The number of cc. of standard bromid solution required to complete the precipitation, multiplied by the value of 1 cc. in terms of acetanilid, will give the quantity of this substance present in the aliquot taken.

COMMENTS AND SUGGESTIONS BY THE REFEREE.

The preliminary or gross separation of acetphenetidin and acetanilid from complex mixtures is materially lengthened, if the preparation contains, in addition to those substances, caffein or antipyrin, or both, in which event it would be necessary to subject the mixture of four ingredients to hot digestion with dilute sulphuric acid in order to convert acetphenetidin and acetanilid to phenetidin and anilin sulphates, respectively, from which caffein and antipyrin could be easily separated by means of chloroform, after which acetphenetidin and acetanilid should be regenerated by treating the aqueous-acid solution of the corresponding sulphates with solid sodium bicarbonate in slight excess, thereupon with a few drops of acetic anhydrid, followed by extraction with chloroform.[1]

In the operation of transferring the acetous solution of acetphenetidin-acetanilid mixture to the graduated flask containing standard iodin, great care must be exercised that none of the dissolved substances crystallize out as such during or after the transfer, either in the liquid or about the neck of the Erlenmeyer, since any undissolved acetphenetidin introduced into the iodin reagent would fail in obtaining its full complement of iodin, thus vitiating the determination. The necessary transfer is effected most conveniently, and indeed without loss, by the use of an Erlenmeyer provided with a lip, a form easily made in the laboratory from the ordinary type by heating the neck of the flask in a moderate blast flame, then by the aid of a file or other suitable instrument pulling down the rim to the desired pitch. Thus modified, the flask delivers aqueous solutions with little or no tendency to run down on the outside of the neck.

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[1] U. S. Bur. Chem. Bull. 162, p. 197.

The standard solution of iodin employed in the foregoing separation and the one giving the best results has a strength slightly above fifth-normal; that is, a solution containing 30 grams of iodin and 40 grams of potassium iodid to the liter. To prepare, dissolve the potassium iodid in the least possible quantity of water, add the iodin and, after complete solution, dilute to 1 liter. Twenty-five cc. of this reagent, the volume taken for each determination, are standardized with a solution of sodium thiosulphate containing 30 grams to the liter, the value of which has been ascertained in turn by titration with very carefully purified iodin. The end point is best observed by adding to the liquid 1 or 2 drops of freshly prepared starch solution toward the close of the titration. In measuring off the standard iodin, make the readings by the aid of transmitted light. This is easily done by holding an electric bulb just back of the burette, thus bringing the lower meniscus into sharp relief.

For this and similar iodometric operations, very pure iodin was prepared by dissolving the commercial resublimed product in concentrated aqueous potassium iodid, pouring the clear liquid into a large volume of water, filtering and washing the finely precipitated iodin on a porous plate several times with water, then drying first in the air and finally in a desiccator over sulphuric acid, where it was kept for future use in a glass stoppered weighing tube. To standardize the sodium thiosulphate, weigh about 0.3 gram of pure iodin in a small glass capsule (about $\frac{1}{2}$ inch high and $\frac{5}{8}$ inch diameter), provided with a closely fitting glass cap or stopper, which, together with capsule and cover, is transferred to a 200 cc. Erlenmeyer, containing 0.5 gram of potassium iodid dissolved in 10 cc. of water. After complete solution, titrate the iodin with sodium thiosulphate, using 1 or 2 drops of starch solution as indicator.

In the qualitative examination of preparations or mixtures, of which acetphenetidin alone is a known or declared ingredient, suitable tests, such as are prescribed in the U. S. Pharmacopoeia or in Allen's Commercial Organic Analysis, should be applied in order to verify the presence or absence of acetanilid. For the identification of acetphenetidin, either alone or in admixture with acetanilid, the following test, in addition to those ordinarily employed for this substance, will be found of value: To 1–2 mg. of the sample in a test tube add a drop of acetic acid, 0.5 cc. of water and 1 cc. of tenth-normal iodin, warm the mixture to about 40°C., then add a drop of concentrated hydrochloric acid. Almost immediately if acetphenetidin alone is present, or on cooling and agitating the liquid, if the sample consists in large part of acetanilid, the iodin addition product of acetphenetidin separates in the form of reddish brown leaflets or needle-like crystals. In the presence of considerable acetanilid, the periodid first separates as minute oily globules, which on vigorous shaking gradually become crystalline aggregates. This test is so delicate that as little as 0.5 mg. of acetphenetidin may be detected, if alone, in the form of its characteristic periodid.

(6) That the following method for the estimation of acetphenetidin (phenacetin) and salol in mixtures,[1] be adopted as provisional:

ACETPHENETIDIN (PHENACETIN) AND SALOL.

(W. O. EMERY, G. C. SPENCER AND C. C. LEFEBVRE METHOD.)

The separation proper follows two distinct lines of procedure, involving, on the one hand, acid hydrolysis of acetphenetidin, on the other, alkaline hydrolysis of salol. In the first instance, a direct estimation of acetphenetidin only is contemplated, the salol being determined by difference in all cases where the combined weight of both constituents is known or can be ascertained conveniently. In this procedure, the acetphenetidin is converted temporarily into phenetidin sulphate and acetic acid, while in the case of salol partial volatilization results during the process of digestion. The phenetidin salt, by virtue of its insolubility in chloroform, is freed readily from further contaminating salol by treatment with that solvent, then by means of acetic anhydrid and sodium bicarbonate reconverted into acetphenetidin, the latter being isolated subsequently and weighed.

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[1] *J. Ind. Eng. Chem.*, 1915, **7**: 681.

In the case of alkaline hydrolysis, the salol is converted first into sodium salicylate and phenolate, from which the acetphenetidin is separated easily and subsequently recovered by extraction with chloroform. The salol is thereupon treated in rotation with standard bromin solution in excess, hydrochloric acid, potassium iodid and sodium thiosulphate, with the net result that 12 atoms of bromin are expended on every molecule of salol. Titration of the iodin liberated by unexpended bromin affords sufficient data for determining the salol content.

Acid Hydrolysis.

Acetphenetidin ($C_2H_5OC_6H_4NHCOCH_3$), *melting point 135°C.*—Weigh, on a small (5.5 cm.) tared filter, an amount of the sample equal to, or a multiple of, the average weight of a unit dose, wash with successive small portions of chloroform (about 40 cc.), sufficient at least to insure complete extraction of all acetphenetidin and salol present in the mixture. Collect the solvent in a 100 cc. tared beaker, and evaporate by means of a blast to apparent dryness. During this operation, the beaker may with advantage be allowed to stand on a warm plate (50°–60°C.) without undue loss of solid substance. Permit the beaker to remain 24 hours in the open at the ordinary temperature, weigh several times until practically constant, then transfer the crystalline residue to a 50 cc. lipped Erlenmeyer by dissolving in and washing with sufficient chloroform, evaporate the solvent by means of a blast and gentle heat, add 10 cc. of dilute sulphuric acid (1 to 10) and digest at full steam-bath heat until the liquid is reduced one half. Add 10 cc. of water and continue the digestion as before, adding finally a second 10 cc. of water and evaporating to 5 cc. as in the two preceding cases. Transfer the residual liquid to a small separatory funnel by pouring and washing with about 20 cc. of water and extract in rotation with 15, 10 and 5 cc. of chloroform, washing each portion as obtained with 5 cc. of water in a second separator, in order to recover traces of phenetidin sulphate possibly taken up by the chloroform, finally rejecting the latter since it contains all the salol not previously eliminated during the process of digestion.

Treat the aqueous-acid solution of phenetidin sulphate in the first separatory funnel, to which likewise the wash water used in the second separator has been transferred, with sodium bicarbonate preliminary to regenerating acetphenetidin, substantially as prescribed in the method for the estimation of caffein and acetphenetidin in mixtures.

Salol ($HOC_6H_4CO_2C_6H_5$), *melting point 42°C.*—Ascertain the quantity of salol present in the sample by subtracting the weight of acetphenetidin, as found above, from the combined weight of the two ingredients determined in the preliminary or gross separation.

Alkaline Hydrolysis.

Acetphenetidin.—Weigh an amount of the sample containing not more than 100 mg. of salol on a small tared filter, exhaust with chloroform as in acid hydrolysis, collecting the solvent in a small lipped Erlenmeyer flask. Add 10 cc. of 2.5 per cent sodium hydroxid solution and heat for 5 minutes on a steam or vapor-bath at the temperature of boiling water. At the end of this period, remove from the bath and cool quickly to room temperature in running water in order to reduce to a minimum any tendency of the acetphenetidin to undergo partial hydrolysis. Transfer the liquid to a separatory funnel by pouring and washing with a minimum of water, finally rinsing out the flask with the first 20 cc. portion of chloroform used in extraction. Extract the aqueous-alkaline solution with three 20 cc. portions of chloroform, washing consecutively each portion as obtained in a second separator with 5 cc. of water, prior to passing the solvent through a small, dry filter into a 200 cc. Erlenmeyer, from which the combined chloroformic portions are distilled to a residue of about 5 cc. Transfer the latter to a small tared beaker or crystallizing dish, by pouring and washing with sufficient fresh chloroform, evaporate on the steam-bath with the additional aid of a blast if available, cool and weigh the residual acetphenetidin at intervals until constant.

Salol.—Unite the aqueous-alkaline solutions from both separatory funnels in a suitable (500 cc.) glass-stoppered bottle, dilute with water to about 200 cc., run in from a burette an excess (about 45 cc.) of seventh-normal potassium bromid-bromate, follow with 10 cc. of concentrated hydrochloric acid, close the flask and shake for 1 minute, then at intervals over 30 minutes. At the end of this time, add 10 cc. of 15 per cent potassium iodid solution, agitating the closed flask at intervals for 15 minutes. Titrate the free iodin with standard thiosulphate (preferably seventh-normal), previously adjusted to the standard bromin solution, 1 cc. of which is equivalent to 0.002548 gram of salol. From the number of cc. of standard bromin solution expended, calculate the salol on the basis of 12 atoms of bromin to 1 molecule of salol.

COMMENTS AND SUGGESTIONS BY THE REFEREE.

In order to facilitate the work and minimize errors due to recharging burettes, seventh-normal solutions are advocated so that the volume of thiosulphate required may not exceed the capacity of a 50 cc. burette. The thiosulphate is standardized against pure iodin. The strength of the bromin solution may also be determined by the aid of pure acetanilid.[1]

(7) That the following method for the estimation of acetanilid and sodium salicylate in mixtures, be adopted as provisional:

ACETANILID AND SODIUM SALICYLATE.

(W. O. EMERY METHOD.)

Weigh on a metal scoop or watch glass an amount of the powdered sample equal to, or a multiple of, an average unit dose, transfer to a separatory funnel containing 10 cc. of water and, for every unit dose, 100 mg. of solid sodium bicarbonate. In the case of coated tablets and pills, take one or more for each determination, first crushing in a mortar prior to treatment in the separator. In the examination of alcoholic preparations, concentrate a measured volume on the steam-bath until the alcohol is expelled, transfer to a separatory funnel with a minimum of water, add sufficient solid sodium bicarbonate (0.5–1.0 gram) and proceed as follows:

Acetanilid (*$C_6H_5NHCOCH_3$*), *melting point 113°C.*—Extract with three 50 cc. portions of chloroform, wash each in rotation with 5 cc. of water in a second separator, and collect the solvent without previous drying in a 200 cc. Erlenmeyer. Distil the chloroform very gently to about 5 cc., add sufficient dilute sulphuric acid and digest on the steam-bath to complete hydrolysis, titrating the anilin sulphate substantially as outlined under estimation of caffein and acetanilid in mixtures.

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[1] U. S. Bur. Chem. Bull. 132, p. 198, footnote b.

Sodium salicylate ($HO.C_6H_4CO_2Na$).—Acidify the aqueous solution of sodium salicylate with a few drops of concentrated hydrochloric acid and extract with three to five 25 cc. portions of chloroform, sufficient at least to exhaust the salicylic acid present in the mixture. Treat each portion in rotation in a second separator with 20 cc. of water containing, for every 100 mg. of salicylic acid known or believed to be present in the sample under analysis, 1 gram of anhydrous sodium carbonate. Shake vigorously and, after clearing, wash each portion again in a second separator with 5 cc. of water, which on completion of this operation is united with the main aqueous-soda solution of sodium salicylate. Dilute to a known volume, withdraw an aliquot, representing about 100 mg. of salicylic acid, to a 200 cc. Erlenmeyer, make up to 100 cc., heat nearly to boiling, then run in from a burette 25–40 cc. of strong (about fifth-normal) iodin solution, sufficient at least to insure an excess of this reagent during digestion of the product for an hour on the steam-bath. Discharge the free iodin with a few drops of thiosulphate solution, decant the clear liquid through a tared Gooch, care being taken that most of the precipitate $(C_6H_2I_2O)_2$, remains in the flask. To the latter add 50 cc. of boiling water, digest for 10 minutes on the steam-bath, filter, gradually washing all the reddish substance into a Gooch, using for this purpose and final washing about 200 cc. of hot water. Dry to constant weight in an air-bath at 100°C. Multiply the weight of the precipitate by the factor 0.4658 to obtain the quantity of sodium salicylate present in the aliquot taken.

COMMENTS AND SUGGESTIONS BY THE REFEREE.

Should the combination contain caffein or antipyrin, or both, these substances will appear with acetanilid in the first chloroformic extraction and require a treatment substantially as outlined on pages 64–65. In the event of replacement of acetanilid by acetphenetidin, the general procedure would not be materially altered, this substance being weighed directly after recovery from its washed chloroformic solution as separated from sodium salicylate. If, instead of sodium salicylate, the combination contains the free acid or its ammonium salt, the analyst should have no difficulty in meeting such conditions by appropriate modifications in detail; for example, the addition of a larger quantity of sodium bicarbonate to insure an excess of this reagent above that required for the production of sodium salicylate prior to extraction with chloroform.

In the analysis of a mixture of caffein, acetanilid, sodium salicylate and codein sulphate, the following essential operations would be appropriate:

(1) Extraction of caffein, acetanilid and salicylic acid from the acidified solution.

(2) Washing the chloroformic solution with aqueous soda solution for the recovery of salicylic acid, preliminary to its treatment with iodin solution.

(3) Separation of caffein and acetanilid as outlined on page 60.

(4) Recovery of codein from the solution of its sulphate after treatment with sodium bicarbonate and chloroform.

(8) That the following method for the estimation of caffein, acetanilid and quinin sulphate in mixtures, be adopted as provisional:

CAFFEIN, ACETANILID AND QUININ SULPHATE.

(W. O. EMERY METHOD.)

The separation of caffein and acetanilid from quinin sulphate is based on the fact that the bisulphate of this alkaloid in aqueous media is practically insoluble in U. S. P. chloroform, while the first named substances are readily taken up by this solvent. The gross separation, therefore, resolves itself into the following main operations:

Transfer to a separatory funnel one or more average unit doses of the powdered (crushed, in the case of pills and coated tablets) sample, follow with 20 cc. of water and 50 cc. of chloroform, add 10 drops of dilute sulphuric acid, then extract in the usual way. After clearing, wash the solvent in a second separator with 5 cc. of water prior to reception in a 200 cc. Erlenmeyer. Repeat the foregoing operations with two 50 cc. portions of chloroform, finally distilling the united solvent by gentle heat to about 10 cc., preliminary to the hydrolytic treatment of the caffein-acetanilid mixture, and subsequent separation and estimation of these two drugs in accordance with the procedure outlined on page 60.

Quinin sulphate, $(C_{20}H_{24}N_2O_2)_2.H_2SO_4 + 7H_2O$.—Unite the wash water used in the second separator with the aqueous-acid solution of quinin bisulphate, add solid sodium bicarbonate in slight excess, then extract with three 50 cc. portions of chloroform, washing each portion in rotation with 5 cc. of water in a second separator, prior to passing through a dry filter into a 200 cc. Erlenmeyer and distillation therefrom to about 5 cc. Evaporate the residual liquid on the steam-bath to apparent dryness, dissolve the amorphous alkaloid in 5 cc. of neutral alcohol and titrate with fiftieth-normal hydrochloric acid (using 2 drops of methyl red solution as indicator) until the yellow color changes to faint red. Remove to steam-bath and heat until most of the alcohol has been expelled. Should the color have reverted to yellow as the result of such treatment, add sufficient acid to just restore the red, note the number of cc. of acid expended and multiply same by the factor 8.66, the number of mg. of quinin sulphate equivalent to 1 cc. of fiftieth-normal hydrochloric acid, to ascertain the quantity of quinin sulphate present in the mixture.

COMMENTS AND SUGGESTIONS BY THE REFEREE.

Since quinin sulphate readily loses a portion of its water of crystallization when exposed to dry air, it follows that the commercial product employed in compounding medicinal preparations may contain an appreciably greater quantity of the alkaloid than is represented by the formula and, finally, that the quantity of the drug as ascertained by analysis may be appreciably in excess of the declaration.

In the event that the mixture contains acetphenetidin in place of acetanilid, the separation of the several ingredients differs in no material way from the procedure just outlined.

(9) That the following method for the estimation of caffein, acetanilid, and codein sulphate in mixtures, be adopted as provisional:

CAFFEIN, ACETANILID AND CODEIN SULPHATE.

(W. O. EMERY METHOD.)

The entire treatment is similar in every way to that prescribed for caffein, acetanilid and quinin sulphate, except that in the case of codein direct weighing of the alkaloid may be resorted to advantageously prior to titration.

Codein sulphate, $(C_{18}H_{21}NO_3)_2.H_2SO_4 + 5H_2O$.—Transfer the chloroformic solution of codein to a small tared beaker by pouring and rinsing with sufficient solvent, evaporate to apparent dryness on the steam-bath, add a few drops of alcohol to the amorphous residue, then a like amount of water and evaporate again, cool finally and weigh the usually crystalline product until constant. The weight of this residue multiplied by the factor 1.3144 will give the quantity of codein sulphate originally present.

In order to check this result volumetrically, dissolve the codein in 3–5 cc. of neutral alcohol and titrate with fiftieth-normal sulphuric acid (using methyl red as indicator), until the yellow color is replaced by a faint red. Multiply the number of cc. of standard acid thus expended by the factor 7.80, the number of mg. of codein sulphate equivalent to 1 cc. of fiftieth-normal acid. The quantity of codein sulphate as found by weight will usually be slightly greater than that determined by titration.

(10) That the following method for the estimation of caffein, acetanilid, quinin sulphate and morphin sulphate, be adopted as provisional:

CAFFEIN, ACETANILID, QUININ AND MORPHIN SULPHATE.

(W. O. EMERY METHOD.)

As in the combination, caffein, acetanilid and quinin sulphate, so also in one containing the additional constituent morphin sulphate, the alkaloidal content is readily separated from the two first-named substances by means of chloroform, while the separation of the alkaloids themselves is based on the ability of morphin to yield a morphinate insoluble in chloroform with a metallic hydroxid. The procedure thus resolves itself into the following particulars:

Caffein and acetanilid.—Transfer to a separatory funnel an amount of the powdered sample equal to, or a multiple of, a unit dose (the quantity of material taken for an individual analysis should not contain less than one-half grain of morphin), add 20 cc. of water and 10 drops of dilute sulphuric acid, then extract with three 50 cc. portions of alcohol-free chloroform, washing each in rotation in a second separator with 5 cc. of water, which is subsequently united with the alkaloidal solution in the first separator. Treat the chloroformic solution of caffein and acetanilid as outlined on page 60.

Quinin sulphate.—Add 4–5 cc. of aqueous sodium hydroxid (5 grams of pure sodium hydroxid in 50 cc. of water), to the solution of quinin and morphin sulphates and extract with four 40 cc. portions of chloroform (U. S. P.), wash each in rotation with 5 cc. of water and pass the clear solvent through a small dry filter into a 200 cc. Erlenmeyer, from which the solvent is later removed by gentle distillation, the residual quinin being titrated with fiftieth-normal hydrochloric acid as already outlined on page 72.

Morphin sulphate, $(C_{17}H_{19}NO_3)_2.H_2SO_4 + 5H_2O$.—Wash the filter employed in the preceding operation with 5 cc. of water, and unite with the aqueous-alkaline solution of the morphinate. Then add 0.5 gram of ammonium chlorid (or an amount slightly in excess of that required to free the morphin as well as convert all of the sodium hydroxid to sodium chlorid) and to the resulting ammoniacal solution add 45 cc. of U. S. P. chloroform and 5 cc. of alcohol, then extract in the usual way, washing the solvent in a second separator with 5 cc. of water. After clearing, pass the chloroform through a small dry filter (as well as a pledget of cotton if deemed necessary) into a 200 cc. Erlenmeyer. Repeat the extraction with three 40 cc. portions of U. S. P. chloroform, wash and filter as before, finally collect all the solvent in an Erlenmeyer and distil therefrom to about 10 cc. Transfer to a small tared beaker by pouring and washing with additional chloroform, evaporate to apparent dryness, add 0.5 cc. each of water and neutral alcohol, induce crystallization by stirring with a glass rod and final evaporation to dryness. Cool and weigh until constant. Check the weight of morphin thus determined by titration with fiftieth-normal sulphuric acid, using a drop of methyl red as indicator. To this end dissolve the alkaloid in 1–2 cc. of warm neutral alcohol, then after solution is complete, add the acid until the color passes from yellow to red. Evaporate most of the alcohol on the steam-bath, and in the event that the color reverts to yellow add just sufficient acid to restore the red. Note volume of acid expended, multiplying the number of cc. by 7.53 (the number of mg. of morphin sulphate equivalent to 1 cc. of fiftieth-normal acid) to ascertain the quantity of morphin sulphate present in the mixture.

COMMENTS AND SUGGESTIONS BY THE REFEREE.

For the purpose in question, alcohol-free chloroform is readily prepared by washing the U. S. P. product repeatedly with water.

In the various operations involving fixation and subsequent liberation of morphin by means of fixed alkali and ammonium chlorid, the most careful attention should be paid to the manner of adding these reagents, since any undue excess of either might nullify the entire procedure. Any large excess of sodium hydroxid would naturally require for its reduction a correspondingly large amount of ammonium chlorid, the latter in turn yielding its pro rata of hydroxid, relatively large quantities of which through interaction with sodium chlorid tend to inhibit any permanent liberation of alkaloid and thus prevent complete extraction. Furthermore, ammonium chlorid in large amount operates retentively on the morphin in solution, due in part possibly to the formation of an alkaloidal hydrochlorid.

Despite all precautions looking to the exclusion of impurities from the morphin as weighed, the amount of this substance thus determined will usually be greater than that found volumetrically. In order to insure the greatest possible accuracy in volumetric operations on alkaloidal residues like quinin, morphin, and codein, it is suggested that whenever possible the strength of the standard acid used be checked by titration against the pure alkaloid under examination.

(11) That the following method for the evaluation of gum tragacanth,[1] be adopted as provisional:

TRAGACANTH.

(W. O. EMERY METHOD.)

A knowledge of the quantity of volatile (acetic) acidity developed in the acid hydrolysis of gum tragacanth (*Astragalus gummifer*) affords a valuable index of the purity of this commodity when compared with results obtained by similar treatment of so-called "Indian gum" (*Cochlospermum gossypium* and *Sterculia urens*), a not uncommon substitute and adulterant of tragacanth. The term "volatile acidity", as here used is the number of cc. of tenth-normal potassium or sodium hydroxid required to neutralize the volatile (acetic) acid obtained by subjecting the products of the action of boiling aqueous phosphoric acid on 1 gram of the gum to distillation with steam. As the result of such treatment of numerous authentic samples of whole and powdered tragacanth, it was found that 3.6–3.7 cc. (averages obtained with 35 samples whole and 21 samples powdered gum, respectively) tenth-normal alkali represents the volatile acidity of this gum, corresponding to 2.15–2.20 per cent of acetic acid, while in the case of Indian gum 26.7–26.5 cc. of tenth-normal alkali (corresponding to 15.97–15.79 per cent of acetic acid) were obtained as averages of 9 samples of whole and 14 samples of powdered gum respectively.

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[1] *J. Ind. Eng. Chem.*, 1912, **4**: 374.

In determining the volatile acidity, the following procedure was used:

Treat 1 gram of the whole or powdered sample in a 700 cc. round-bottomed flask, provided with a long neck, for several hours in the cold with 100 cc. of distilled water and 5 cc. of sirupy phosphoric acid until the gum is completely swollen. Boil gently for 2 hours in connection with a reflux condenser, whereby a nearly clear, colorless solution is effected. A very small amount of cellulose substance will remain undissolved. Then subject the hydrolyzed product to slow distillation in a vigorous current of steam until the distillate amounts to 600 cc. and the acid residue to about 20 cc. This should not be driven too far, however, otherwise there may be danger of scorching the non-volatile, organic degradation products, with consequent possible contamination of the distillate. It has been found that a spray trap of the form shown in U. S. Bur. Chem. Circ. 94, p. 4, if used in connection with the flask containing the hydrolyzed gum, is effective in preventing traces of phosphoric acid being carried over into the distillate. Titrate with tenth-normal potassium hydroxid in connection with 10 drops of phenolphthalein solution, finally boiling the liquid under examination until a faint pink color persists. Run a control on the same amount of distillate obtained by a parallel operation, with the omission of the gum, but using like quantities of other ingredients and observe the same conditions as in the test.

It should be noted in this connection that while tragacanth yields a practically colorless solution when boiled with aqueous phosphoric acid, Indian gum, on the other hand, gives a pink or rose colored solution, a sure indication in an unknown sample that the last named product is present.

REPORT ON SYNTHETIC PRODUCTS.

By W. O. Emery (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

During the past year, coöperative work on synthetic products has been restricted to mixtures containing two drugs, caffein and antipyrin. On account of similarity in behavior towards the more common immiscible solvents, a quantitative separation depending on any simple extraction method was considered impracticable. Accordingly, recourse was had to a procedure based on the fact that, when an aqueous solution of iodin and sodium bicarbonate is permitted to react with antipyrin, either alone or in the presence of caffein, mono-iodoantipyrin is formed. This latter substance, together with caffein, is thereupon extracted with chloroform, the weight of extractives ascertained and the iodin therein estimated as silver iodid, from which the antipyrin can be calculated by means of the appropriate factor. This procedure was very carefully tested under a variety of conditions prior to submission to the collaborating chemists, hence there was every reason to expect satisfactory results from workers of average skill in the art.

The following instructions were sent to collaborators:

COÖPERATIVE WORK.

CAFFEIN AND ANTIPYRIN IN ADMIXTURE.

PREPARATION OF SAMPLE.

Powder No. 16.—Prepared by triturating anhydrous caffein and antipyrin (1 to 4) in a mortar and subsequent sifting.

DETERMINATION.

Weigh on a metal scoop or watch glass 0.25 gram of the sample, transfer to a 150 cc. separatory funnel with 5 cc. of alcohol-free chloroform and 10 cc. of water, add 0.5 gram of sodium bicarbonate and about 10 cc. of fifth-normal iodin (or double the quantity of tenth-normal iodin), then shake vigorously for 1 minute. After this operation a slight excess of iodin should still be apparent in the liquid menstruum, provided all the antipyrin has been converted to iodoantipyrin. If, however, all the iodin has been thus expended, a little more should be added and the mixture again shaken. Then discharge the uncombined iodin by means of a small crystal of sodium thiosulphate, add 20 cc. of U. S. P. chloroform and shake. After clearing, draw off the solvent into a second separator containing 5 cc. of water, shake and after clearing pass the chloroform through a small, dry filter into a 50 cc. tared beaker, evaporate to apparent dryness on the steam-bath, accelerating this operation by means of an air blast, if available. Repeat the extraction with two (three, in case tenth-normal iodin were used) 25 cc. portions of chloroform, washing, filtering and evaporating each portion in rotation substantially as directed for the first portion. Recover all traces of crystalline products separating about the tips of funnels and edge of the filter by judicious washing with chloroform. Dry the final colorless, crystalline residue of caffein and iodoantipyrin for 30 minutes at 100°C., then cool and weigh. Designate this weight *A*.

Dissolve this residue in 5 cc. of glacial acetic acid, add 10 cc. of saturated aqueous solution of sulphur dioxid, then transfer the resultant liquid to a 400–500 cc. beaker by pouring and rinsing with hot water until the final volume amounts to about 200 cc. Add aqueous silver nitrate solution (containing about 0.25 gram of silver nitrate) and follow with a few drops of nitric acid, then heat nearly to boiling, stirring the while in order to agglomerate the silver iodid. Add about 15 cc. of concentrated nitric acid, cover the beaker with a watch glass and boil gently for 5 minutes. Filter by decantation through a tared Gooch, wash the precipitate twice with boiling water, finally transferring the silver iodid completely to the Gooch, washing several times with hot water and finally with alcohol. Dry for 30 minutes in an air-bath at 110°C., cool and weigh.

The weight of silver iodid multiplied by the factor 0.8012 yields the quantity of antipyrin present in the sample taken. The quantity of caffein present in the sample is ascertained by subtracting the product, obtained by multiplying the weight of silver iodid by the factor 1.3374, from the weight designated *A*.

PREPARATION OF SAMPLE.

Liquid No. 17.—Obtained by dissolving 5 grams of anhydrous caffein and 25 grams of antipyrin in water and diluting to 1 liter at 25°C. The liter flask employed was standardized at 20°C.

DETERMINATION.

By means of a pipette standardized at 20°C., transfer 10 cc. of the solution, previously warmed or adjusted to 25°C., to a 150 cc. separatory funnel containing 0.5 gram of sodium bicarbonate. Add about 15 cc. of fifth-normal iodin (or double the quantity of tenth-normal iodin), then shake vigorously for 1 minute. All succeeding operations are identical with those outlined for the powder mixture.

Report recoveries in mg. and parts per 100.

The foregoing procedure is based on the fact that, when an aqueous solution of iodin and sodium bicarbonate is permitted to react with antipyrin, whether alone or with other substances, such as caffein, monoiodoantipyrin is formed. This latter substance, together with caffein, is thereupon extracted with chloroform, the weight of such mixture ascertained and the iodin therein estimated as silver iodid, from which the antipyrin is calculated by means of the appropriate factor. In view of the somewhat radical treatment with dilute nitric acid, to which the caffein is necessarily subjected incidental to the precipitation of silver iodid, a direct determination of that substance is impracticable. The quantity of caffein is, however, ascertained by subtracting the amount of iodoantipyrin, as calculated from the silver iodid, from the combined weight of caffein and iodoantipyrin.

The use of alcohol-free chloroform is specified in order to preclude the possible formation of iodoform, the presence of which would necessarily render the method valueless.

The final washing of the silver iodid with alcohol should be thorough in order to eliminate certain unidentified organic substances arising from the action of the nitric acid on the antipyrin complex.

DISCUSSION OF RESULTS.

Examination of the following tabulated data reveals the fact that a majority of the collaborators were able to obtain quite satisfactory results. A few, however, report values so divergent from the theory as to indicate that somewhere in the procedure the directions may not have been sufficiently clear to insure uniformity in manipulation. Wherever such discrepancy is material, low antipyrin and high caffein values were found almost without exception, unquestionably due for the most part to incomplete iodation. Assuming all apparatus used to have been strictly clean and chemicals pure, further contributory causes for antipyrin shortage and caffein excess may have been due to the following causes: (a) Failure to properly desiccate or remove all volatile substances from the composite residue *A;* (b) incomplete removal of alcohol-soluble material associated with the silver iodid; or, (c), faulty technique.

Coöperative results on the recovery of caffein and antipyrin in admixtures.

ANALYST	POWDER, NO. 16		LIQUID, NO. 17	
	Antipyrin 80.00 per cent	Caffein 20.00 per cent	Antipyrin 83.33 per cent	Caffein 16.67 per cent
J. M. Bartlett, Orono, Me.	70.88	28.72	81.47	17.03
	70.36	27.56	78.80	21.43
L. A. Brown, Lexington, Ky.	79.35	22.44	82.10	23.63
	78.96	22.96	83.37	25.87
E. O. Eaton, San Francisco, Cal.	80.92	18.96	82.80	17.30
	.....	.....	83.00	16.87
W. O. Emery, Washington, D. C.	79.96	19.96	82.97	16.77
	79.76	20.28	83.27	16.73
	79.96	20.04	82.93	17.17
C. O. Ewing, Washington, D. C.	.....	.....	83.43	16.40
	.....	.....	83.10	16.77
	.....	.....	83.37	16.37
J. F. Darling, New York, N. Y.	73.04	28.04	82.33	22.73
	79.56	15.44	81.17	18.50
	79.99	20.12	82.87	17.80
	.....	.....	82.13	18.67
	.....	.....	83.97	16.93
H. Engelhardt, Baltimore, Md.	79.80	19.24	83.50	20.00
	79.80	19.24	83.60	18.96
	79.16	21.48	82.27	20.84
	79.56	21.36	82.80	20.56
H. C. Fuller, Washington, D. C.	79.44	22.44	82.60	17.85
	79.16	22.56	82.63	17.53
C. K. Glycart, Chicago, Ill.	78.44	19.76	82.53	17.37
	78.76	21.08	82.67	17.07
	79.04	20.64	.....	.....
A. W. Hansen, Honolulu, Hawaii	73.32	26.60	81.27	20.33
	77.84	22.56	80.50	20.73
R. Hertwig, Honolulu, Hawaii	73.00	26.12	78.50	21.10
	77.24	24.04	.....	.....
	79.04	22.24	.....	.....
	79.00	21.12	.....	.....
	79.08	21.60	.....	.....
	79.08	23.20	.....	.....
	79.64	21.52	.....	.....
H. B. Mead, Philadelphia, Pa.	79.08	21.40	83.60	17.93
	80.48	19.84	83.50	17.57
	79.88	27.04	83.57	19.13
	80.16	21.32	82.70	18.60
C. B. Morison, New Haven, Conn.	77.32	23.32	83.00	16.73
	77.68	22.84	.....	.....
	79.09	22.76	.....	.....
	79.40	22.08	.....	.....

Coöperative results on the recovery of caffein and antipyrin in admixtures.—Continued.

ANALYST	POWDER, NO. 16		LIQUID, NO. 17	
	Antipyrin 80.00 per cent	Caffein 20.00 per cent	Antipyrin 83.33 per cent	Caffein 16.67 per cent
S. Palkin, Washington, D. C.	80.12	19.88	83.33	17.10
	79.76	20.56	83.17	16.97
	.....	.....	83.23	16.43
G. C. Spencer, Washington, D. C.	79.16	20.63	83.03	17.33
	79.28	21.06	82.56	17.93
	79.40	20.60	82.50	18.06
A. R. Todd, Lansing, Mich.	74.67	28.40	81.27	20.20
	74.12	29.00	81.17	19.87
	73.90	28.16	80.97	20.27
	.....	.....	80.33	18.00
	.....	.....	80.50	18.37
C. D. Wright, Washington, D. C.	79.00	21.40	82.90	17.00
	79.40	21.20	82.97	17.17
	79.40	20.80	.....	.....

COMMENTS.

L. A. Brown suggested a greater number of extractions with chloroform since in his opinion caffein and iodoantipyrin continue to be recovered even up to the eighth or ninth extraction.

J. F. Darling recommended that in order to insure a more complete desiccation of the composite residue *A*, the time of heating be extended one-half hour.

H. Engelhardt facilitated the final expulsion of chloroform from this residue by the addition of a little ether prior to heating in the air-bath.

H. C. Fuller suggested the employment of a third separator for the assembling of all three (or more) chloroformic portions prior to passing through cotton into the tared beaker.

R. Hertwig and *A. W. Hansen* recommended washing the silver iodid once by decantation with alcohol after first decanting off the hot aqueous solution.

The *referee* suggests the following procedure if it seems desirable to recover most of the chloroform:

> Withdraw the several portions into a 200 cc. Erlenmeyer instead of the tared beaker, distil most of the solvent by gentle heat, then transfer the residue to the tared beaker by pouring and washing with sufficient fresh chloroform for treatment as specified in the method.

The following committee on revision of the constitution was appointed: William Frear, of Pennsylvania; R. J. Davidson, of Virginia; and E. W. Magruder, of Virginia.

DETERMINATION OF SANTONIN IN LEVANT WORMSEED.

By E. K. Nelson (Bureau of Chemistry, Washington, D. C.).

In view of certain difficulties reported in the determination of santonin, an investigation of the various methods in use was undertaken, experiments being conducted with the Thaeter method, the Katz method, and Fromme's modification of the Katz method.

The Katz and Katz-Fromme methods necessitate the use of a solubility correction factor which is avoided in the Thaeter method. With the latter method, however, all of the santonin was not recovered, and as it is altogether cumbersome and tedious it was dropped from further consideration.

The chief difficulty found with the Katz-Fromme method was that the santonin residues were not free from resinous impurities, and were consequently discolored and returned a santonin value in excess of the truth. Accurate checks were not obtained on account of the incomplete elimination of impurities.

With certain precautions in its use the Katz method, with slight modifications, was found to give concordant and evidently accurate results. The santonin residues were of good color, and the method is very simple and easy to operate.

SUGGESTED MODIFICATION OF THE KATZ-FROMME METHOD.

Extract 10 grams of medium ground Levant wormseed in a Soxhlet extraction apparatus for 3 hours with chloroform. Distil off the chloroform till 7–8 grams remain, add 100 grams of a 5 per cent solution of barium hydroxid, and heat on the steam-bath till the odor of chloroform has disappeared. Boil for 5 minutes, cool and pass carbon dioxid (washed through sodium bicarbonate solution to free it from traces of acid which might be carried over mechanically) till saturated. An excess of carbon dioxid will do no harm. Filter by suction, preferably on a small Büchner funnel, and wash twice with 10 cc. of water. To the filtrate, heated on a steam-bath, add 5 cc. of 25 per cent hydrochloric acid and warm for 5 minutes. Cool till lukewarm and extract with 20, 15 and 15 cc. of chloroform, passing the solvent through a small filter into a flask. Evaporate to dryness, being careful to see that the last traces of chloroform are removed. Add 7.5 grams of absolute alcohol to the residue, and dissolve (by gentle warming if necessary). Then add 42.5 grams of water heated to 60°–70°C., stopper the flask and allow to cool. (At this point it is advisable to see that crystallization starts, which can be done by scratching the side of the beaker or adding a *minute* crystal of santonin. Samples put aside in a cool place for 24 hours, and which contained santonin in liberal amount have been found in a supersaturated condition where this precaution was not observed.) Keep the flask at a temperature of 15°–17°C. for 24 hours. Filter and wash with 2 portions of 10 cc. of 15 per cent alcohol by weight. Dry the flask and filter at 100°C., dissolve the santonin left in the flask in chloroform and filter into a tared beaker. Wash the santonin out of the flask and filter thoroughly with chloroform, evaporate the filtrate and dry at 100°C. till all traces of chloroform are gone. To the weight found add 0.04 gram for the santonin dissolved in the dilute alcohol, and multiply the total by 10 for the per cent of santonin.

(The solubility factor was found correct for a temperature of 15°–17°C. At 30°–32°C., 0.054 gram of santonin was left in solution.)

The above method was submitted to a number of chemists for cooperative work and criticism, with a uniform sample of Levant wormseed. The results obtained are as follows:

Results of coöperative work on santonin.

ANALYST	SANTONIN	ANALYST	SANTONIN
	per cent		*per cent*
C. K. Glycart, Chicago, Ill.	2.44	J. G. Roberts, Philadelphia, Pa.[1]	2.96
	2.44		2.91
	2.52		
		H. C. Fuller, Washington, D. C.	2.81
J. F. Darling, New York, N. Y.	2.77		3.11
P. Rothberg, New York, N. Y.	2.74	J. M. Francis, Detroit, Mich.	2.52
			2.75
J. Ehrlich, New York, N. Y.	2.65		
		H. Engelhardt, Baltimore, Md.	2.79
H. Runkel, St. Louis, Mo.	3.02		2.83
	2.89		2.77
B. H. St. John, Washington, D. C.	2.85	E. K. Nelson, Washington, D. C.	2.87
			2.66
H. M. Rhea, Washington, D. C.	2.62		2.61
	2.58		2.69
	2.62		2.58
	2.48		2.73
Average			2.72
Maximum			3.11
Minimum			2.44

[1] The solubility factor used was 0.054. When the factor 0.04 was used these results became 2.82 per cent and 2.77 per cent.

COMMENTS BY ANALYSTS.

J. G. Roberts, of Smith, Kline & French: Although somewhat elaborate, the method submitted is satisfactory. It gives concordant results and yields santonin in a crystalline state, apparently free from resin and having only a little color. However, there is one feature that may operate against the general adoption of the method and that is the requirement that crystallization shall be conducted at 15°–17°C. for 24 hours. We consider that 2°C. is a rather limited range to maintain for 24 hours. We consider also that the method would be improved if instructions were given to have the diluted alcohol used for washing the crystals at the same temperature as the solution containing the crystals of santonin. In order to eliminate as much error as possible we filtered and washed the santonin crystals at 30°C. and used the correction of 0.054 gram given in the method for that temperature.

H. C. Fuller: In both determinations by the Nelson method the identical procedure was used, but the second assay contained some resin and was darker than the santonin in the first sample.

J. F. Darling: The chloroform extraction appears to be more efficient than the extraction with ether, and the residues obtained by the modified method are much cleaner than in the method of J. Katz which we have hitherto used.

J. M. Francis: We do not think that 3 hours' extraction in a Soxhlet is sufficient for the thorough extraction of the drug. In the first assay the extraction was continued to 3½ hours, the distillation of the chloroform being moderately rapid throughout the process. In the second case the extraction was allowed to proceed for about an hour and the heat was then shut off for the night, the drug being left well covered with chloroform in the Soxhlet extractor. In the morning the chloroform in the Soxhlet was very deeply colored, and the extraction was continued for 3 hours longer in the usual way.

We are of the opinion that there is a distinct advantage in Fromme's original process of maceration with occasional shaking for 12 hours or more, and then of removing an aliquot portion of the chloroformic solution for the assay.

A 5 per cent solution of barium hydroxid is directed. Owing to the limited solubility a 5 per cent solution can only be obtained by warming to a temperature of 30°–40°C. If this strength is desired, therefore, specific instructions should be included, directing that the solution be warmed.

On the whole, we consider that the process works very well, and doubtless with a little practice results would be obtained which would check better than those indicated above.

H. Engelhardt: The santonin obtained was by no means pure and colorless, the color varying from light brown to dark brown. Some time ago I made quite a number of assays of samples of Levant wormseed and I have found that by the Katz-Fromme method (which is rather convenient inasmuch as no Soxhlet is used for extracting the drug) reliable and uniform results could be obtained, the santonin being in most cases very pure. I applied this method to the powder submitted and obtained 2.67 per cent and 2.70 per cent, respectively, which correspond fairly well with those obtained by the other method. The santonin was of a creamy white, thus showing that only a very small percentage of impurities was present.

H. Runkel: I wish to state that these residues were exceptionally pure considering the fact that there is a large amount of resinous material, etc., which must be removed. I find, however, that boiling with barium hydrate for a full 5 minutes is quite essential.

No report was presented by the associate referee on medicated soft drinks.

A paper on the "Comparison of the Physical Properties of Some Official Volatile Oils with Special Reference to the Requirements of the U. S. P., 1910," by Frank Rabak of the Bureau of Plant Industry, was read by Mr. Rabak. This article has since been published in the *J. Am. Pharm. Assoc.*, 1914, **3**: 670.

No report was made by the associate referee on alkaloids.

REPORT ON BALSAMS AND GUM RESINS.

By E. C. Merrill (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

The object of this work was to secure data which would indicate the extent to which the lead number method for asafoetida would check when used by independent chemists working on samples of known character. It was further desired to verify the efficacy of certain color tests for foreign resins which are later bulletined in the method.

PREPARATION OF SAMPLES.

For these purposes a large sample of good asafoetida, about 10–12 pounds, was procured. This was run through a power sausage grinder several times in order to thoroughly mix the samples. The friction of the sample passing through the machine warmed the product to a temperature of about 110°F. when it became a semi-fluid mass, easily stirred, and homogenous. This sample was divided into two portions, *A* and *B*. Sample *A* was transferred to $\frac{1}{4}$ pound tins.

Two and ten-hundredths pounds of powdered rosin were added to the remainder of the sample, 5.25 pounds, and the latter passed through the grinder several times to insure a uniform mixture. The amount of adulterant in this case was about 28.5 per cent. This mixture was put up similarly in quarter pound tins and marked *B*.

Sets of Samples *A* and *B* accompanied the instructions sent to collaborators.

ASAFOETIDA.

LEAD NUMBER.

(*1*) *Preparation of Resin.*

Use a sample (about 20 grams) sufficient to furnish between 5 and 10 grams of the ether purified resin. Determine the alcohol insoluble material in the usual manner. Transfer the first 2 filtrates, representing the major part of the sample, to a casserole or a flat-bottomed porcelain dish, and evaporate the alcohol on the steam-bath. Treat the resinous mass with ether (sometimes it is necessary to warm gently to facilitate solution of the resin). Filter the ethereal solution into a separatory funnel and wash with water until the aqueous layer separates without any milkiness. (If the ether solutions persist in remaining turbid, more ether may be added, or it may become necessary to dry the ether solution by shaking with sodium chlorid in the separatory funnel after as much as possible of the aqueous solution has been removed.) Then filter the ethereal solution through a folded filter paper, moistened with ether, into a flask or beaker, and evaporate the solvent on the steam-bath.

(*2*) *Determination.*

(The residual ether purified resin from the above preparation is now in a state where it can usually be broken up when cold, and powdered.)

Into a small tared beaker (about 75 cc. capacity) weigh roughly about 1.1–1.2 grams of the resin prepared in (*1*) and dry for 5 hours in the air-bath at 110°C. Place in a desiccator, cool and weigh. (The weight noted is to be used in subsequent calculations.) Dissolve in 20 cc. of 95 per cent alcohol, boil gently until the resin is in solution, transfer to a 100 cc. graduated flask, wash the beaker with hot 95 per cent alcohol, care being taken that the final volume does not exceed 70 cc. Add 25 cc. of the alcoholic lead acetate (*A*) agitate and allow to stand overnight. Make up to the mark with 95 per cent alcohol, shake well, allow to stand a few minutes, and then filter through a fluted paper and pipette an aliquot of 25 cc. from the filtrate into a beaker. Add 10 cc. of water and evaporate to 10 cc. Add 5 cc. of 10 per cent sulphuric acid and then 100 cc. of alcohol, stirring vigorously to dissolve any separated resin, and heat if necessary. Allow to stand for an hour, then filter the lead sulphate on a tared Gooch, wash with alcohol and finally with ether, dry at 100°C. to a constant weight and weigh.

Run a blank on the alcoholic lead acetate solution, and calculate the amount of metallic lead absorbed by 1 gram of the dried resin. The mg. of lead per gram of sample represent the lead number. The factor for the conversion of lead sulphate to metallic lead equals 0.6830.[1]

QUALITATIVE TESTS FOR PURITY.

(1) *Copper Acetate.*

Transfer about 40 cc. of the alcoholic solution (obtained by determining the alcohol insoluble) to a separatory funnel, add 30 cc. of petroleum ether, mix thoroughly, then add 50 cc. of water and shake for 30 seconds. Allow to separate and discard the aqueous milky layer. Wash the petroleum ether layer with water until the wash water and petroleum ether are both clear. Add 30 cc. of $\frac{1}{2}$ per cent copper acetate solution and shake well. No green color should appear in the petroleum ether. The appearance of a green color indicates the presence of foreign resins.

(2) *Concentrated Hydrochloric Acid.*

Transfer 3–5 cc. of the alcoholic solution (obtained by determining the alcohol insoluble) to a small beaker, add concentrated hydrochloric acid, drop by drop, until a faint turbidity appears on agitation, then add sufficient alcohol to clear up the turbidity. A green color results which fades within 10 minutes. The appearance of a red or violet color indicates the presence of galbanum or other foreign resins. (The temperature at which this reaction takes place should be kept below 35°C.)

(3) *Ferric Chlorid.*

To 1 or 2 cc. of the alcoholic solution diluted to 5 cc. with 95 per cent alcohol, add a few drops of ferric chlorid solution (10 per cent). A clear, green color results. The appearance of a red tinge or a dark red or a brownish color indicates sophistication with foreign resins.

REMARKS AND COMMENTS BY COLLABORATORS.

Earl B. Putt: When the acid is first added in the hydrochloric acid test the green coloration is very pronounced, but this color soon fades and a variety of color shades are produced when alcohol is added in varying amounts. Therefore, this test does not appear to be especially indicative. The ferric chlorid test is quite satisfactory and seems to require a rather strong solution of ferric chlorid. The test is improved rather than interfered with by an excess of ferric chlorid.

The copper acetate test is very satisfactory. In giving directions for the above qualitative tests it might be well to state in each case the approximate weight of the sample represented in the volume of alcoholic solution taken. Different analysts will use different quantities of alcohol in determining the alcohol soluble portion, hence concentration of the alcoholic solution will vary.

H. Runkel: The concentrated hydrochloric acid test with *B* does not fade like *A*.

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[1] For the preparation of alcoholic lead acetate dissolve 5 grams of normal lead acetate in 20 cc. of water and add 80 cc. of 95 per cent alcohol. A turbidity generally results, due to the precipitation of lead carbonate caused by carbon dioxid in the alcohol. Allow the solution to stand overnight. The clear, supernatant liquid can then be used without filtering for the determination of the lead number.

The blank on 25 cc. of alcoholic lead acetate solution should be equivalent to at least 1 gram, calculated as $PbSO_4$.

Lead number and color tests for foreign resins obtained on Samples A and B.

ANALYST[1]	LEAD NUMBER		COPPER ACETATE TEST *A*	CONCENTRATED HYDROCHLORIC ACID TEST *A*	FERRIC CHLORID TEST *A*
	A	*B*			
E. O. Eaton, San Francisco, Cal.	200.0	109.0	O.K.	O.K.	O.K.
H. Kleuter, Madison, Wis......	233.2	160.0	Light green	Violet	Brownish
E. C. Merrill, Washington, D. C.	240.4	151.1	Negative	Green, fading gradually	Green
E. B. Putt, New York, N. Y....	238.0	165.0	Negative	Green, changing to blue-green	Green
S. Palkin, Washington, D. C...	245.6	158.8	O.K.	O.K.	O.K.
P. Rothberg, New York, N. Y.	241.0	164.0	Negative	Green to pinkish brown	Yellowish green
J. K. Rippetoe, New York, N. Y.	220.1	144.9	Negative	Blue green	Yellow green
N. Smith, New York, N. Y.....	213.1	140.1	....	....	....
H. Runkel, St. Louis, Mo.......	201.0	147.9	Negative	Green fading in 3 minutes	Clear green
H. A. Seil, New York, N. Y....	240.1	154.7	Negative	Green to light pink	Green
A. E. Taylor, San Juan, P. R...	207.0	166.1	Negative	Green, fading to very light pink	Clear olive solution
J. F. Darling, New York, N. Y.	237.7	143.2	Negative	Faintly positive	Negative
E. Clark, Boston, Mass........	237.5	164.7	Negative	Reddish brown to violet tinge	Positive
F. M. Boyles, Baltimore, Md...	184.0	104.0	Negative	....	....
J. B. Luther, New York, N. Y.	245.6	151.6	Negative	Green, fading to faint red	Green
H. B. Mead, Philadelphia, Pa..	222.6	144.1	Faint green	....	....

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[1] The associate referee desires to acknowledge his indebtedness to the analysts whose names appear in this column for the assistance rendered in this work.

Color tests obtained on Sample B.

ANALYST[1]	COPPER ACETATE TEST	CONCENTRATED HYDROCHLORIC ACID TEST	FERRIC CHLORID TEST
E. O. Eaton, San Francisco, Cal...	Resins present	Resins present	Resins present
H. Kleuter, Madison, Wis..........	Deep green	Not red or violet	Brownish
E. C. Merrill, Washington, D. C....	Intense green	Green, quickly changing to dark yellow	Brownish yellow
E. B. Putt, New York, N. Y........	Bright green	Green	Red
S. Palkin, Washington, D. C........	Resin indicated (green)	Positive	Positive
P. Rothberg, New York, N. Y......	Strongly positive	Green to brown	Brown
J. K. Rippetoe, New York, N. Y....	Green	Grayish	Reddish brown
H. Runkel, St. Louis, Mo...........	Decided green	Faint green	Decided red tinge
H. A. Seil, New York, N. Y........	Strongly positive (green)	Green to dark yellow	Brownish
A. E. Taylor, San Juan, P. R.......	Green	Green, fading to yellow	Brown solution, slightly turbid
J. F. Darling, New York, N. Y.....	Strongly positive	Negative	Positive
E. Clark, Boston, Mass............	Positive	Negative	Positive
F. M. Boyles, Baltimore, Md.......	Decided green	....	....
J. B. Luther, New York, N. Y.....	Intense green	Green, fading to colorless	Yellowish brown
H. B. Mead, Philadelphia, Pa......	Strong green	....	....

[1] The associate referee desires to acknowledge his indebtedness to the analysts whose names appear in this column for the assistance rendered in this work.

COMMENTS BY THE ASSOCIATE REFEREE.

It is believed, in view of the results obtained by the other collaborators that the lead numbers by Messrs. Eaton and Boyles are abnormally low and are probably due to misinterpretation of the method at some stage in the process. The figures obtained by Messrs. Runkel and Taylor on Sample *A* also appear somewhat below the average. In the knowledge of the associate referee, Messrs. Eaton, Putt, Rothberg, Seil, Darling and Clark were previously familiar with this method and there appears to be a general concordance in the results obtained by them.

The general average of the lead numbers on *A*, excepting the 4 previously mentioned, which appear to be abnormal, is 234.2. The widest variation from this figure is roughly about 10 per cent in one instance, while the remainder of the figures differs by about 5 per cent or less. The general average of the lead number for *B*, not taking into account 2 results which appear abnormal, is 154.0, with a maximum variation from this figure of less than 5 per cent.

The copper acetate test for colophony appears satisfactory.

The concentrated hydrochloric acid test appears of doubtful value as an index for foreign resins.

The ferric chlorid test in the hands of different chemists leads to varying conclusions and is therefore doubtful.

SUMMARY OF RECOMMENDATIONS.

It is recommended—

(1) That the lead number test for asafoetida be adopted unofficially by the association, and that the method be further studied during the coming year.

(2) That further work be done to establish more satisfactory color tests for foreign resins.

(3) That the iodin number of cinnamein in Peru balsam as an index to true or artificial Peru balsam be studied during the coming year with a view to later collaboration.

REPORT OF COMMITTEE ON REVISION OF CONSTITUTION.

By Wm. Frear (State College, Pa.), *Chairman.*

The resolution introduced by Mr. Lythgoe having been considered, it is recommended that Paragraph 2 be amended by the insertion after the first sentence of the following:

> Analytical chemists connected with municipal laboratories that perform work upon any of the subjects specified in Paragraph 1 hereof, shall be entitled ex officio to associate membership, with the privilege of discussion but without those of entering motions, voting or becoming eligible for office.

Approved.

REPORT OF COMMITTEE ON RECOMMENDATIONS OF REFEREES.[1]

By P. F. Trowbridge (Experiment Station, Columbia, Mo.), *Chairman.*

It is believed that the present system of referees and the assigning of special subjects to associate referees for investigation is the best policy for the association.

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[1] Presented by B. B. Ross.

The committee therefore recommends that the executive committee be authorized to appoint additional associate referees to investigate special problems, when in their opinion there is need for such special investigation.

Adopted.

Mr. Frear moved that a vote of thanks be given the President, which was unanimously carried.

The association adjourned.

OFFICERS, REFEREES AND COMMITTEES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, FOR THE YEAR ENDING NOVEMBER, 1915.

Honorary President.

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President.

C. H. Jones, Burlington, Vt.

Vice-President.

R. N. Brackett, Clemson College, S. C.

Secretary-Treasurer.

C. L. Alsberg, Washington, D. C.

Additional Members of the Executive Committee.

Julius Hortvet, St. Paul, Minn.
E. F. Ladd, Fargo, N. Dak.

Referees.

Phosphoric Acid: L. S. Walker, Amherst, Mass.
Nitrogen:
  Determination: R. N. Brackett, Clemson College, S. C.
  Separation of nitrogenous substances: A. W. Bosworth, Geneva, N. Y.
Potash: E. E. Vanatta, Columbia, Mo.
Soils: J. W. Ames, Wooster, Ohio.
Dairy Products: L. I. Nurenberg, Boston, Mass.
Feeds and feeding stuffs: G. L. Bidwell, Washington, D. C.
Food adulteration: Julius Hortvet, St. Paul, Minn.
Sugar: C. A. Browne, New York, N. Y.
Insecticides: R. C. Roark, Washington, D. C.
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Medicinal plants and drugs: W. O. Emery, Washington, D. C.
Water: W. W. Skinner, Washington, D. C.
Testing chemical reagents: C. O. Ewing, Washington, D. C.
Water in foods and feeding stuffs: W. J. McGee, Washington, D. C.
Organic and inorganic phosphorus in foods, feeding stuffs, and drugs: F. M. Beegle, Wooster, Ohio.

Associate Referees.

Phosphoric acid: W. J. Jones, Jr., Lafayette, Ind.
Nitrogen:
  Determination: H. D. Haskins, Amherst, Mass.
  Special study of Kjeldahl method: I. K. Phelps, Washington, D. C.
  Separation of nitrogenous substances:
    Milk and cheese: Leroy S. Palmer, Columbia, Mo.
    Meat proteins: P. F. Trowbridge, Columbia, Mo.

Potash:
  Determination: T. D. Jarrell, College Park, Md.
Soils:
  Nitrogenous compounds: C. B. Lipman, Berkeley, Calif.
Dairy products: J. T. Keister, Washington, D. C.
Feeds and feeding stuffs: A. C. Summers, Columbia, S. C.
  Feed adulteration: Carleton Cutler, W. Lafayette, Ind.
  Crude fiber: C. K. Francis, Stillwater, Okla.
Food adulteration:
  Colors: W. E. Mathewson, New York, N. Y.
  Saccharine products: F. L. Shannon, Lansing, Mich.
  Fruit products: P. B. Dunbar, Washington, D. C.
  Wine: B. G. Hartmann, Chicago, Ill.
  Beer: H. S. Paine, Washington, D. C.
  Distilled liquors: A. B. Adams, Washington, D. C.
  Vinegar: C. M. Bradbury, Richmond, Va.
  Flavoring extracts: A. E. Paul, Chicago, Ill.
  Spices: H. E. Sindall, Philadelphia, Pa.
  Baking powder: H. E. Patten, Washington, D. C.
  Meat and fish: E. D. Clark, Philadelphia, Pa.
  Fats and oils: R. H. Kerr, Washington, D. C.
  Dairy products: Julius Hortvet, St. Paul, Minn.
  Cereal products: L. A. Fitz, Manhattan, Kans.
  Vegetables: W. D. Bigelow, Washington, D. C.
  Cocoa and cocoa products: H. C. Lythgoe, Boston, Mass.
  Tea and coffee: J. M. Bartlett, Orono, Me.
  Preservatives: A. F. Seeker, New York, N. Y.
  Heavy metals in foods: E. L. P. Treuthardt, Washington, D. C.
Sugar: M. N. Straughn, Washington, D. C.
Insecticides: O. B. Winter, East Lansing, Mich.
Inorganic plant constituents: R. W. Thatcher, St. Paul, Minn.
Medicinal plants and drugs:
  Synthetic products: W. O. Emery, Washington, D. C.
  Medicated soft drinks: W. F. Sudro, Fargo, N. Dak.
  Medicinal plants: Frank Rabak, Washington, D. C.
  Alkaloids: H. C. Fuller, Washington, D. C.
  Balsams and gum resins: E. C. Merrill, Washington, D. C.
  Pepsin: V. K. Chestnut, Washington, D. C.
Water: H. P. Corson, Urbana, Ill.
Testing chemical reagents: C. S. Lykes, Clemson College, S. C.

PERMANENT COMMITTEES.

Coöperation with Other Committees on Food Definitions.

William Frear, State College, Pa., *Chairman.*
Julius Hortvet, St. Paul, Minn.
J. P. Street, New Haven, Conn.

Recommendations of Referees and Revision of Methods.

(Figures in parentheses refer to year in which appointment expires.)
P. F. Trowbridge, *Chairman.*

SUBCOMMITTEE A: A. J. Patten (1918), W. W. Skinner (1916), *Chairman, Bureau of Chemistry, Washington, D. C.*, B. B. Ross (1920). (Phosphoric acid, nitrogen, potash, soils, inorganic plant constituents, insecticides, water.)

SUBCOMMITTEE B: R. E. Stallings (1918), *Chairman, Department of Agriculture, Atlanta, Ga.*, P. F. Trowbridge (1916), H. C. Lythgoe (1920). (Dairy products, feeds and feeding stuffs, sugar, water in foods and feeding stuffs, organic and inorganic phosphorus in foods, feeding stuffs, and drugs, separation of nitrogenous substances, testing chemical reagents, medicinal plants and drugs.)

SUBCOMMITTEE C: L. M. Tolman (1918), H. E. Barnard (1916), *Chairman, State Board of Health, Indianapolis, Ind.*, R. E. Doolittle (1920). (Food adulteration.)

SPECIAL COMMITTEES.

Editing Methods of Analysis (U. S. Bur. Chem. Bull. 107, Rev.).

R. E. Doolittle, New York, N. Y., *Chairman.*
W. A. Withers, Raleigh, N. C.
J. P. Street, New Haven, Conn.
A. F. Seeker, New York, N. Y.
G. W. Hoover, Chicago, Ill.
B. L. Hartwell, Kingston, R. I.

Proposed Journal of Agricultural Chemistry.

C. H. Jones, Burlington, Vt., *Chairman.*
R. N. Brackett, Clemson College, S. C.
C. L. Alsberg, Washington, D. C.
Julius Hortvet, St. Paul, Minn.
E. F. Ladd, Fargo, N. Dak.

Availability of Phosphoric Acid in Basic Slag.

C. B. Williams, West Raleigh, N. C., *Chairman.*
C. G. Hopkins, Urbana, Ill.
H. D. Haskins, Amherst, Mass.
B. L. Hartwell, Kingston, R. I.
J. A. Bizzell, Ithaca, N. Y.

Review of the Analysis of Lime-Sulphur Solutions.

R. J. Davidson, Blacksburg, Va., *Chairman.*
C. S. Cathcart, New Brunswick, N. J.
H. H. Hanson, Orono, Me.

OFFICERS, REFEREES, ASSOCIATE REFEREES AND COMMITTEES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, FOR THE YEAR ENDING NOVEMBER, 1916.

Honorary President.

H. W. **Wiley**, Woodward Building, Washington, D. C.

President.

R. N. **Brackett**, Clemson College, S. C.

Vice President.

J. K. **Haywood**, Bureau of Chemistry, Washington, D. C.

Secretary-Treasurer.

C. L. **Alsberg**, Bureau of Chemistry, Washington, D. C.

Additional Members of the Executive Committee.

W. J. **Jones**, Jr., La Fayette, Ind.
E. B. **Holland**, Amherst, Mass.

Referees.

Phosphoric acid: W. J. Jones, Jr., La Fayette, Ind.
Nitrogen:
  Determination: H. D. Haskins, Amherst, Mass.
  Separation of nitrogenous substances: L. L. Van Slyke, Geneva, N. Y.
Potash: E. E. Vanatta, Columbia, Mo.
Soils: J. W. Ames, Wooster, Ohio.
Dairy products: Harry Klueter, Dairy and Food Department, Madison, Wis.
Foods and feeding stuffs: A. C. Summers, Department of Agriculture, Commerce and Industries, Columbia, S. C.
Food adulteration: Julius Hortvet, Dairy and Food Commission, St. Paul, Minn.
Sugar: C. A. Browne, Sugar Trade Laboratory, New York, N. Y.
Insecticides: R. C. Roark, Bureau of Chemistry, Washington, D. C.
Inorganic plant constituents: A. J. Patten, East Lansing, Mich.
Medicinal plants and drugs: W. O. Emery, Bureau of Chemistry, Washington, D. C.
Water: W. W. Skinner, Bureau of Chemistry, Washington, D. C.
Water in foods and feeding stuffs: W. J. McGee, U. S. Custom House, Savannah, Ga.
Testing chemical reagents: C. O. Ewing, Bureau of Chemistry, Washington, D. C.
Organic and inorganic phosphorus in foods, feeding stuffs and drugs: E. B. Forbes, Wooster, Ohio.
Lime requirements of soils: F. P. Veitch, Bureau of Chemistry, Washington, D. C.

Associate Referees.

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  Special study of Kjeldahl method: I. K. Phelps, Bureau of Chemistry, Washington, D. C.
Separation of nitrogenous substances:
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  Meat products: P. F. Trowbridge, Columbia, Mo.
Potash:
  Determination: T. D. Jarrell, College Park, Md.
Soils:
  Nitrogenous compounds: C. B. Lipman, Berkeley, Cal.
Dairy products: J. T. Keister, Bureau of Chemistry, Washington, D. C.
Foods and feeding stuffs: P. H. Smith, Amherst, Mass.
  Feed adulteration: Carleton Cutler, W. La Fayette, Ind.
  Crude fiber: C. K. Francis, Stillwater, Okla.
Food adulteration:
  Colors: W. E. Mathewson, Bureau of Chemistry, Washington, D. C.
  Saccharine products: (not appointed).
  Fruit products: P. B. Dunbar, Bureau of Chemistry, Washington, D. C.
  Wine: B. G. Hartmann, Transportation Building, Chicago, Ill.
  Beer: H. S. Paine, Bureau of Chemistry, Washington, D. C.
  Distilled liquors: A. B. Adams, Bureau of Internal Revenue, Washington, D. C.
  Vinegar: E. H. Goodnow, Federal Office Building, Minneapolis, Minn.
  Flavoring extracts: A. E. Paul, Transportation Building, Chicago, Ill.
  Spices: H. E. Sindall, Weikel & Smith Spice Co., Philadelphia, Pa.
  Baking powder: H. E. Patten, Bureau of Chemistry, Washington, D. C.
  Meat and fish: E. D. Clark, 1833 Chestnut Street, Philadelphia, Pa.
  Fats and oils: R. H. Kerr, Bureau of Animal Industry, Washington, D. C.
  Dairy products: Julius Hortvet, Dairy and Food Department, St. Paul, Minn.
  Cereal products: J. A. LeClerc, Bureau of Chemistry, Washington, D. C.
  Vegetables: W. D. Bigelow, National Canners Association, Washington, D. C.
  Cocoa and cocoa products: E. Bloomberg, Federal Building, Buffalo, N. Y.
  Tea and coffee: H. M. Loomis, Eastport, Me.
  Preservatives: A. F. Seeker, U. S. Appraiser's Stores, New York, N. Y.
  Metals in foods: David Klein, State Food Commission, Chicago, Ill.
Sugar: M. N. Straughn, Bureau of Chemistry, Washington, D. C.
Insecticides: O. B. Winter, East Lansing, Mich.
Inorganic plant constituents: R. W. Thatcher, University Farm, St. Paul, Minn.
Medicinal plants and drugs:
  Synthetic products: W. O. Emery, Bureau of Chemistry, Washington, D. C.
  Medicated soft drinks: W. W. Skinner, Bureau of Chemistry, Washington, D. C.
  Medicinal plants: A. Viehoever, Bureau of Chemistry, Washington, D. C.
  Alkaloids: H. C. Fuller, Institute of Industrial Research, Washington, D. C.
  Balsams and gum resins: E. C. Merrill, Bureau of Chemistry, Washington, D. C.
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PRESIDENT'S ADDRESS.[1]

THE STORAGE OF SUGAR IN THE MAPLE TREE.

By C. H. Jones (Agricultural Experiment Station,
Burlington, Vt.), *President.*

Maple sugar is obtained mainly from the species of maple known to botanists as *Acer saccharum.* Census returns indicate a total yearly production in the United States of the equivalent of some 45,000,000 pounds. The more northern sections contribute most largely to the supply. The yield is subject to extreme variations, being dependent on certain daily weather conditions during a few weeks in early spring. Although insignificant in amount when compared with the world's production of cane and beet sugars, yet as a table luxury maple products have long held an honored place among our food products.

ECONOMIC INVESTIGATIONS.

Economic investigations connected with the production of maple sugar and sirup have occupied the attention of many students of the subject and their observations have been recorded in various publications of a scientific and popular nature. They include data on the history of the maple industry, composition and flow of maple sap, the action of micro-organisms on sap and their effect on the finished product, together with a vast amount of strictly chemical data directed toward a proper standardization of the marketable product and the detection of adulteration therein.

Chemically speaking, the sugar from the maple has been shown to be identical in composition with the ordinary granulated sugar secured from the cane and beet. The importance it holds as a delicacy is due to a characteristic and pleasing flavor associated with it which, however, is destroyed if the usual methods of sugar refining are applied to the crude product.

The nature of the considerations about to be presented is fundamental rather than economic; fundamental in that they deal with the tree itself and seek to indicate the various changes taking place within the trunk at different seasons of the year and their visible manifestations as indicated by pressure and sap flow. Before proceeding further a brief description of maple wood will be given. No attempt is made to describe the several structures in detail but attention is directed to such tissues as seem most directly concerned with our general subject.

[1] Presented Tuesday, November 16, as special order of business for 11.30 o'clock.

GENERAL STRUCTURE.

In general structure, the maple trunk or stem (*xylem*) may be divided into a brown heart wood (*duramen*) and a white sap wood (*alburnum*). The sap wood contains the living cells and is the main conducting medium for water and food. The heart wood is relatively lifeless and functions largely as a mechanical support for the crown and as a reservoir in which to store water during the winter months.

The stem is composed of three kinds of cells, viz., wood fibers, wood parenchyma and vessels, without intercellular spaces. At right angles to these cells, extending from the circumference toward the heart, are the *medullary rays*. These are *living cells*. They vary greatly in width and length and serve as the main centers for the storage of starch. Measurements of the medullary rays in the maple have shown that they occupy about 18% by volume of the outer or recently formed sap wood.

The starch grains found in the maple are angular, not uniform in shape, and vary in size from 1.4–5.7 microns in diameter. The characteristic blue color is obtained by treatment with iodin solution. Starch is stored not only in the medullary rays but also in the wood (parenchyma) which in some cases forms pockets, visible to the naked eye. These as a rule extend at right angles to and border on the medullary rays. Starch may also be found in the rows of cells (from three to many) formed in the summer wood of the past few years.

Between the medullary rays are longitudinal cells often of great length containing water and food ready for transference throughout the tree. They are known as *tracheae*. Associated with these are similar but shorter cells known as *tracheids*. Both *tracheae* and *tracheids* are *dead cells*. They comprise about 7% by volume of the sap wood.

CHEMICAL PHASES OF SUGAR STORAGE.

A consideration of the more strictly chemical phases of sugar storage in the maple will indicate conditions existing within the tree during the year. The specimens used in determining this matter were second growth maples, quite uniform as to size, age and leaf area, which grew near each other, under similar general conditions of soil formation and exposure. Differences due to these causes were thus minimized and as samples were taken monthly for 29 months a period is represented including 3 successive sugar seasons, 2 full periods of growth and 3 periods of rest. To further confirm these observations precautions were taken during the second 12 months to secure 2 trees on the dates which the first year's work indicated to be what may be termed critical points in the yearly cycle.

The several trees were felled, measured and immediately brought to the laboratory where suitable sections representing the base (outer and inner wood) and the top wood were reduced to sawdust by means of a circular saw. The samples thus prepared were subjected to chemical analysis directed primarily to ascertain the content of moisture, sugar, and starch, which are assumed to be the so-called readily available stored foods. The last transformation of the sugar molecule used for plant food purposes is outside a strictly chemical province, being rather a problem for the plant physiologist.

While variations in the results obtained for corresponding months in different years were noted, and individual differences were occasioned by samples and natural fluctuations in soil moisture, sunlight and temperature conditions, yet the same general trend of all results was plainly manifested during successive seasons.

Instead of burdening you with a mass of data representing monthly conditions for 29 months the results have been summarized in a brief table where the months of the year are divided into 4 groups, each group representing a distinct epoch in the life functions of the tree.

The first division includes the months of December, January and February, the winter months, during which interval the tree is generally considered dormant as regards development. The second division comprises the months of March and April. The sap flow occurs during this

TABLE 1.

Percentage of available carbohydrate food in maple wood.

DETERMINATION	DECEMBER, JANUARY AND FEBRUARY	MARCH AND APRIL	MAY, JUNE, JULY AND AUGUST	SEPTEMBER, OCTOBER AND NOVEMBER
OUTER BASE WOOD:				
Water	35.01	32.86	30.30	29.80
Sucrose	0.94	0.80	0.36	0.72
Reducing sugars	1.40	0.53	0.13	0.41
Starch	0.78	0.96	1.01	1.43
INNER BASE WOOD:				
Water	30.28	29.64	26.87	29.05
Sucrose	0.49	0.52	0.18	0.41
Reducing sugars	0.65	0.33	0.09	0.26
Starch	0.43	0.49	0.62	0.51
TOP WOOD (outer and inner):				
Water	33.85	31.36	32.46	32.14
Sucrose	1.12	1.04	0.41	0.83
Reducing sugars	1.79	0.67	0.23	0.59
Starch	1.09	1.33	1.23	1.63
ROOTS:				
Water	33.07	35.46	31.00	30.23
Sucrose	1.52	0.93	0.66	1.41
Reducing sugars	1.00	0.79	0.42	0.32
Starch	3.92	2.25	3.41	5.76

period which, in northern latitudes, is designated the sugar season. The tree is preparing for its yearly work of growth and reproduction. The third period, May, June, July and August, represents the time of bud and leaf development, the formation of the annual ring and the storage of reserve food. The fourth division, September, October and November, is a period of gradual preparation for the cold of winter. Early in this period, as the leaves fall, reserve foods are deposited largely in the form of starch which later may be changed in part to sugar.

SUCROSE IN TRACHEAE AND TRACHEIDS.

When fresh maple wood is taken from a tree, for example during March or April, suitably pulverized, and treated with water, the resulting solution is found to contain both reducing sugars and sucrose. Maple sap as it flows from the tree has been shown repeatedly to be practically free from reducing sugars, but to contain sucrose. This would indicate that there is a separation of the two sugar solutions in the tree.

By taking a suitable section of maple wood and subjecting it to a water pressure of about 30 pounds, it was found that only sucrose was removed, no reducing sugars being washed out. The amount of sucrose thus obtained depended to some extent on the quantity of water forced through the stem. Three trials on different samples using 1250, 900 and 2500 cc. of water removed 28, 51 and 67% respectively of the total sucrose present. It would seem, therefore, that sucrose must be stored chiefly in the *tracheae*, the long longitudinal tubes, to which mention has been made, while the reducing sugars must be contained in the cell sap of the living cells.

CONSIDERATION OF RESULTS.

From a study of Table 1 it is clear that marked variations exist in the water, sugar and starch content of wood in different parts of the maple tree at similar periods and also that the composition is not constant during the year. Further, that the top wood is supplied more abundantly with sugars and starch than either the outer or inner base wood.

Considering the results obtained in the periods indicated, it is noted that the amount of water present is greatest in the outer, inner and top wood sections during the cold winter months, December to February. Similarly, the sugars are also higher. The starch, on the other hand, is less at this time than at other intervals during the year. The percentage of water is lowest in the inner wood but if account is taken of the total bulk of wood represented a large excess of water would be found in the inner, over that contained in the outer, base tissues.

The March-April period shows a slight reduction in the water content of the several sections. The sugar percentages continue to decrease as warmer weather approaches, while the starch content increases.

The third period, representing the season of growth, indicates that the amount of water in the tree is at its minimum, large amounts having been given off by transpiration. The percentage amount of sugars present is small but it remains quite uniform during the growing season. The starch content is practically unchanged in the outer and top wood, while the inner tissues show an increase. The small amount of sugars present during this period of active photosynthesis is explained first, by the fact that these constituents have been drawn on to feed the developing buds and leaves and, secondly, by the fact that the elaborated food is used immediately in the development of the annual ring.

The last period, September to November, finds the water content of the outer and top wood remaining constant while a decided gain in moisture is observed in the inner section. The sugars show an increase of some 130% over amounts present during the growing season. In like manner the starch percentages have become augmented in the outer and top sections.

The roots exhibit the highest water content in March and April, the highest sugar content from December to February and the lowest between May and August. The starch content of the roots greatly exceeds that found in the trunk wood regardless of season. It is most abundant during the autumn months, September to November, when an average percentage of 5.76 was obtained. This gradually decreases during the cold winter months and is lowest during the period just preceding the renewal of spring activities when but 2.25% was indicated.

RELATION OF SUCROSE TO REDUCING SUGARS.

There is a condition shown by the results in Table 1 to which special attention should be directed and that is the relation between the percentages of sucrose and reducing sugars and the relation of both to the starch content. Reducing sugars are greater in amount than is sucrose during only one period of the year, December to January. Sucrose predominates throughout the remaining months. The change in relativity begins just previous to the sugar season, March and April. Coincident with the decrease in reducing sugars an increase in starch percentage is observed. The roots throughout the year show a predominance of sucrose over reducing sugars, particularly in the autumn months of September, October and November. Mention already has been made of the large starch content.

DENSITY OF SAP SOLUTIONS.

Investigators are most familiar with the composition of maple sap, secured during March and April by the ordinary tapping process, from that part of the tree designated in Table 1 as outer base wood. It is commonly known that maple sap is essentially a dilute solution of sucrose containing small amounts of mineral matter and that its sugar content varies from 1–7% with an average of approximately 3%. Reference to Table 1 shows that but 0.80% of sucrose was present in the baswood. This amount, however, must have been held in solution by the water in the tree trunk, as were the reducing sugars, and a simple calcue lation will show the sucrose content of the sap to have been 2.34%. This represents an average for several young trees during 3 successive sugar seasons.

The grouping of averages by periods in Table 1 does not emphasize the high concentrations obtained on certain dates. Thus, when the individual tree data are consulted, instances are not lacking which show that this concentration during the sugar season ran as high as 5% of sucrose and that even a total sugar concentration of 12% occurred during extremely cold weather.

In order that the density of the sugar solutions found in the tree may be readily compared the results already tabulated have been calculated to a concentration or density basis.

TABLE 2.

Percentage density of sugar solutions in maple wood.

DETERMINATION	DECEMBER, JANUARY AND FEBRUARY	MARCH AND APRIL	MAY, JUNE, JULY AND AUGUST	SEPTEMBER, OCTOBER AND NOVEMBER
OUTER BASE WOOD:				
Sucrose	2.52	2.34	1.17	2.33
Reducing sugars	3.75	1.55	0.42	1.33
INNER BASE WOOD:				
Sucrose	1.56	1.71	0.66	1.38
Reducing sugars	2.07	1.08	0.33	0.87
TOP WOOD (outer and inner):				
Sucrose	3.05	3.14	1.24	2.47
Reducing sugars	4.87	2.03	0.69	1.76
ROOTS:				
Sucrose	4.27	2.50	2.06	4.41
Reducing sugars	2.81	2.12	1.31	1.00

The same general variations already discussed are again in evidence but these figures more nearly represent the density of the sucrose and reducing sugar solutions found in the cells and tissues composing the tree structure. Starch, being insoluble in water, is not included in this calculation although one would be justified in so including it for it is well known that starch is one of the reserve foods stored by the maple and that it must be transformed into soluble sugars before it can be transported.

DENSITY OF SAP INCREASES FROM BASE TO TOP OF TREE.

The ascent of water and food to the tops of tall trees has long occupied the attention of scientists. No explanation, entirely satisfactory, thus far has been presented although many theories have been advanced. Data as to the amounts of soluble sugars and water in different parts of the tree undoubtedly have a bearing on this question. The increased carbohydrate concentration in the top wood of the maple over that in the base section has been noted. For several reasons, particularly when the structure of the wood and region of consumption of food are considered, it seems logical to assume that this increase in concentration of the sugars, together with the higher starch content, should be a gradual one, as we ascend the tree.

A small maple tree cut early in May was examined in sections of 4 feet each, from the base to the top, with the following results:

TABLE 3.

Percentage density of sugar solutions.

SECTION	WATER	STARCH	REDUCING SUGARS	SUCROSE	TOTAL SUGARS
1st (base)..........	29.07	0.83	0.10	1.82	1.92
2nd...............	33.15	0.81	0.21	1.78	1.99
3rd...............	28.97	0.92	0.37	2.26	2.63
4th...............	26.85	1.06	0.32	2.92	3.24
5th (top)..........	25.84	1.10	0.30	3.10	3.40

Table 3 would seem to indicate that the sugar concentration of both active cell, and *tracheae*, sap gradually increases from the lower portions of the tree trunk upward. Starch also shows a similar increase. On the other hand, water, in this instance, decreases in amount as we ascend.

INTERPRETATION OF RESULTS.

It is a relatively easy matter to present for consideration data secured by physical and chemical operations in the field and laboratory but the significance of such data when applied to the solution of nature's processes is not so readily formulated. A lengthy interpretation of the results bearing on the phenomena of sugar formation and sap flow will not be attempted but a few general deductions enumerated.

The work of numerous investigators has shown clearly that sunlight, leaf area and soil moisture are factors influencing the abundant storage of starch and sugars. A correlation table, showing relationships between stored carbohydrates on the one hand, and sunlight, leaf area and rainfall on the other, undoubtedly would give most suggestive results. It is a different matter to prove the relationship from a practical or economic standpoint as weather conditions at the time of sap flow seriously affect results. In other words, one can determine with reasonable certainty that an abundance of carbohydrates has been stored in the tree but the best of prophecies fail when attempts are made to predict the amount of sugar likely to be secured the following spring.

The yearly life cycle of the maple tree has for its objects nutrition and reproduction. Green leaves manufacture starch daily in sunlight. At night it is transformed to soluble carbohydrates and carried down the inner bark and cambium layer where it is again deposited in the medullary rays as starch or remains in solution in the tree tissues. The excess only is thus deposited, what is necessary being used for the formation of the annual ring.

When the leaves fall in the autumn a protective enzyme action may take place in the medullary rays, and sucrose is ultimately formed and distributed through the *tracheae.* The increased concentration of the sugar solutions in the maple trunk during the cold weather period appears to have for its purpose the protection of the tree tissue from freezing, so that an early transference of abundant water and food materials to the leaf-bearing sections of the tree may be established. The maple is the earliest of northern trees to develop its buds and leaves in spring.

The rate of movement of sap in the tapped tree has been measured by injecting lithium chlorid and noting the time of its appearance in the sap from holes at known distances above and below the point of insertion. A rate of 2–6 inches per minute was observed. Since this flow is an artificial one, stimulated nevertheless by conditions within the tree, it does not follow that sap movement up and down within the untapped tree trunk, before leaf formation has started, parallels these rates. Doubtless the water movement is far less rapid, although there is warrant for asserting that changes in sugar concentration and water content in different sections of the tree are taking place constantly.

Considerable work has already been done touching the matter of direction of sap flow and pressure under the artificial conditions created by the ordinary tapping process. It has been shown that during certain weather conditions in early spring, characterized by a sharp frost at night and a rise in temperature to a few degrees above the freezing point the following day, a state of pressure is manifested in the tree trunk and sap is exuded at the tap hole or point of least resistance. This pressure may vary from 1–25 pounds and while the flow of sap is not necessarily in direct proportion to the pressure, there is strong evidence nevertheless that some relation exists. During the greater part of the year a state of negative pressure or suction is exerted by the tree.

The simple determination of the water content of maple wood at different seasons of the year is not without interest. It is more than a coincidence and doubtless for a definite purpose that the highest water content always precedes the leaf formation and transpiration period and that the lowest amount is present when the leaves have ceased their activities.

Beginning in December, the reducing sugars exceed the sucrose content of maple wood until March when a decided decrease occurs in the percentage of reducing sugars. A relatively high percentage of sucrose obtains until May. A predominance of sucrose over reducing sugars, though in reduced amounts, is maintained through the summer and fall, until, in December, the reducing sugars again take the lead. Thus a high sugar content is found during the coldest period, reducing sugars predominating over sucrose, the ratio of reducing sugars, starch and sucrose being 1.5 : 0.8 : 1. As warm weather approaches the total sugar content decreases and a most pronounced increase of sucrose over reducing sugars occurs, the above ratio becoming 0.7 : 1.2 : 1.

Warm weather favors the deposition of starch from soluble carbohydrates. Continued cold weather causes the insoluble starch to decrease in amount, transforming it to soluble carbohydrates, thus increasing the total sugar content during the winter months.

From the chemical evidence presented it is plain that changes are taking place within the tree throughout the year. A fall deposition of starch occurs. Later, as cold weather approaches, the leaf-manufactured food that has been deposited as starch is changed in the living protoplasmic cells to reducing sugars and sucrose which, held in a water solution, fill the vessels throughout the tree. These sugars are most abundant in the young living tissues of the trunk nearest the cambium layer.

Reducing sugars and starch seem to be most intimately associated with the living protoplasmic cells, while a considerable portion at least of the elaborated sucrose fills the dead vessels. These channels, it is well established, serve as quick carriers for water and sucrose to upper and distant parts of the tree, and are in direct communication with the roots or water-gathering system. The uniformity of sap concentration during the four months comprising the growing season indicates that the leaf elaborated carbohydrates are used, either directly or indirectly, for the development of the annual ring. Until that duty is performed no marked increase in soluble carbohydrate storage is noted.

OUR JOURNAL.

In conclusion I desire to say a few words on the subject of our new JOURNAL. The problems confronting us and the ways and means for dealing with them were ably considered by my predecessor in his annual address one year ago. As you all know this association now has a quarterly publication and our deliberations, printed in a most attractive form, will soon be placed promptly at our disposal. This publication is not a one man volume and every member should be allowed and encouraged to do his share. It is needless for me to urge the members to subscribe but I do want to ask particularly that the younger members not only subscribe but also read and study the JOURNAL with a view toward detecting shortcomings and rectifying them in the future.

It is too early to predict the ultimate outcome of our venture. We have men enough, brains enough and, I hope, a loyalty to our chosen profession sufficient to carry on this project.

PROCEEDINGS OF THE THIRTY-SECOND ANNUAL CONVENTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1915.

FIRST DAY.

MONDAY—MORNING SESSION.

The thirty-second annual convention of the Association of Official Agricultural Chemists was called to order by the President, C. H. Jones, of Burlington, Vt., on the morning of November 15, 1915, at the Raleigh Hotel, Washington, D. C.

REPORT ON WATER.

THE SEPARATION AND DETERMINATION OF CALCIUM AND STRONTIUM.

By W. W. Skinner (Bureau of Chemistry, Washington, D. C.), *Referee.*

As a result of the experimental work reported by the referee last year, the Stromeyer-Rose method for the separation and determination of calcium and strontium was modified by substituting for cold water heretofore used a 1% solution of ammonium oxalate for washing the precipitate of the mixed oxalates. As shown by Holleman, Kohlrausch, Richards and others, the solubility of calcium oxalate in cold water and also in hot water is so low that it may be disregarded as a factor of accuracy in ordinary analytical work. The error in the determination of calcium, due to the solubility of the oxalate, should not be in excess of 1 mg. of calcium when the quantity of precipitate is such that 200 cc. or less of wash water are required.

Strontium oxalate, however, as shown by the experimental work reported last year, is quite soluble in hot water and sufficiently soluble in cold water to cause serious error in the determination of strontium. This is particularly true when relatively small amounts of strontium are to be separated from large amounts of calcium, requiring a greater amount of washing of the precipitate. The method was further modified by providing for 2 extractions of the mixed nitrates with the ether-alcohol mixture, which increased the quantity of solvent used.

The method as modified is as follows:

DETERMINATION OF CALCIUM AND STRONTIUM.

Calcium.

Combine the 2 filtrates obtained from the precipitates of iron and aluminium, concentrate to 150–200 cc. and to this solution, containing not more than 0.6 gram of calcium, calculated as calcium oxid, or 1 gram of magnesium, calculated as magnesium pyrophosphate, add 1–2 grams of oxalic acid and sufficient hydrochloric acid to clear the solution. Heat to boiling and neutralize with ammonium hydroxid, stirring constantly. Add ammonium hydroxid in slight excess and allow to stand for 3 hours in a warm place. Filter off the supernatant liquid and wash the precipitate once or twice by decantation with 1% ammonium oxalate solution. Dissolve the precipitate in hydrochloric acid, dilute to 100–200 cc., add a little oxalic acid, and precipitate as above. After standing for 3 hours, filter, wash with the ammonium oxalate solution as before, dry, ignite, heat over a blast lamp, and weigh as calcium and strontium oxids. Subtract from this weight the weight of strontium oxid equivalent to the strontium sulphate found. The difference is the weight of calcium oxid. Calculate to calcium.

As a check on the calcium oxid, evaporate to dryness the combined filtrates from the strontium nitrate, dissolve the calcium nitrate in water, precipitate as oxalate, filter, wash, ignite and weigh as calcium oxid.

Strontium.

Dissolve in dilute nitric acid the oxids obtained in the determination of calcium and test with the spectroscope for strontium. If strontium is present, transfer the nitric acid solution to a small Erlenmeyer flask. Evaporate nearly to dryness over a low flame and heat in an air bath at 150°–160°C. for 1 or 2 hours after the water is evaporated. Break up the dried material with a stirring rod, add 10–15 cc. of a mixture of equal parts of absolute alcohol and ether to dissolve the calcium nitrate. Cork the flask and allow to stand with frequent shaking for 2 hours or longer. Decant the solution through a 5.5 cm. filter, preserving the filtrate. Wash the residue several times by decantation with small portions of ether-alcohol solution. Dry the residue and the filter paper and wash the filter paper repeatedly with small portions of hot water, collecting the filtrate in the flask containing the main portion of the strontium nitrate residue. Add 1 or 2 drops of dilute nitric acid, evaporate, dry, pulverize, and treat with 10–15 cc. of ether-alcohol mixture as before. Cork the flask and let stand for about 12 hours with occasional shaking. Filter, wash with ether-alcohol mixture until a few drops of the filtrate evaporated on a watch glass leave practically no residue. Dry the paper and precipitate. Dissolve the strontium nitrate in a few cc. of hot water. Add a few drops of sulphuric acid, then a volume of alcohol equal to the volume of the solution and allow to stand for 12 hours. Filter, ignite, weigh as strontium sulphate and calculate to strontium. Test spectroscopically for absence of calcium.

A copy of this method, together with the sample specially prepared for the purpose, was sent to 10 chemists who had signified a desire to undertake the coöperative work and reports have been received from 6 of them.

The solution prepared for the coöperative work contained in the aliquot taken for examination 203 mg. of calcium and 21.8 mg. of strontium.

Results of coöperative work on calcium and strontium.

ANALYST	CALCIUM BY DIFFERENCE	CALCIUM DIRECT	STRONTIUM
	Mg. in 10 cc.	*Mg. in 10 cc.*	*Mg. in 10 cc.*
H. P. Corson, Illinois State Water Survey, Urbana, Ill.	200.9	198.6	21.9
O. B. Winter, Agricultural Experiment Station, East Lansing, Mich.	204.6 203.9	197.1 196.1	17.1 16.9
E. L. Griffin, Department of Agriculture, Washington, D. C.	203.2 203.5 203.4	201.5 200.1 203.2	18.1 18.1 17.9
P. L. Hibbard, Agricultural Experiment Station, Berkeley, Cal.	203.2 202.3 202.8 202.9	lost lost lost 201.8	20.0 21.9 21.0 21.0
J. W. Sale, Bureau of Chemistry, Washington, D. C.	203.9 203.8 202.8 203.7	202.6 203.2 202.9 202.7	19.1 18.8 19.0 19.6
W. F. Baughman, Bureau of Chemistry, Washington, D. C.	204.1 203.7 204.3	202.1 202.1 201.9	19.3 20.1 20.3
Maximum	204.6	203.2	21.9
Minimum	200.9	196.1	16.9
Average	203.3	201.1	19.4
Theory	203.0	203.0	21.8

By referring to the table it will be noted that the average of the 17 results reported for calcium by difference varies from theory by only 0.3 mg. or 0.15%; while the difference between the highest and the lowest result is only 3.7 mg. These results are as good as could be expected and confirm the observations of the referee upon the results reported in 1913 to the effect that the method for the separation of calcium from strontium and the determination of calcium by the indirect method leaves little to be desired. The calcium direct is slightly low, the average for the 14 results reported being 1.9 mg. or approximately 0.93% below theory. The cause of the low results may be due to an imperfect extraction by the ether-alcohol mixture of the calcium nitrate from the mixed calcium and strontium nitrates but, if so, it apparently does not affect the final result for strontium.

The results reported for strontium while uniformly slightly lower than theory are fairly satisfactory. The results reported by the 6 analysts at this time are fairly concordant and much superior to the results reported in 1913. The work this year and that of previous years shows conclusively that the separation of the calcium and strontium nitrates by the 2 or more treatments with the ether-alcohol mixture is quite satisfactory, and also that the modified method, substituting a 1% ammonium oxalate solution for cold water in the washing of the mixed oxalates, has eliminated the error in the determination of strontium due to the solubility of strontium oxalate in washing. And while it must be admitted the method gives slightly low results for strontium, due probably to the fact that strontium does not completely precipitate as oxalate,—considerably less so than does calcium,—the results of the investigation and study of the method extending over a period of 4 years are such as to warrant the Association in adopting the method as official and it is so recommended by the referee. The method is presented for its first reading for final adoption as provided in the By-Laws of the Association.

DISCUSSION.

Mr. Skinner: The Committee on Editing Methods of Analysis, Mr. Doolittle, Chairman, early in the year asked the referee to review the methods for the analysis of water. This review showed our methods to be incomplete in that certain standard methods in general use had never been proposed for coöperative work by any referee and had therefore never been adopted as official or provisional methods by the Association, and furthermore, that certain new methods had been substituted for older methods that were not at the same time repealed. It appeared advisable, since the methods were to be printed in the JOURNAL, that such standard methods be included for the sake of completeness, even though they had not been adopted by the Association. These methods were inserted therefore and described as "tentative" to distinguish them from the official and provisional methods. The determinations involved are: turbidity, color, odor, oxygen required (when the chlorin content of the sample is high), dissolved oxygen, specific gravity, hydrogen sulphid, free carbon dioxid, temporary hardness, total hardness, permanent or non-carbonate hardness.

It seemed equally advisable to omit from the JOURNAL those methods which have been superseded, but which have not been officially repealed by the Association. The methods involved are the Pettenkofer method for carbon dioxid and the ammonium sulphate method for barium and strontium.

Under the direction of the referee some time has been devoted to a study of methods for the determination of the radioactivity of waters. The referee has asked Mr. Furber of the Water Laboratory of the U. S. Bureau of Chemistry to present a brief statement of the method and the work.

REPORT ON THE DETERMINATION OF RADIOACTIVITY.

By F. B. FURBER (Bureau of Chemistry, Washington, D. C.).

The Water Laboratory of the U. S. Bureau of Chemistry has been concerned for some time with the examination of substances claimed to be radioactive, chiefly in connection with the enforcement of the Federal

Food and Drugs Act. The samples have been the so-called "radioactive" bottled mineral waters and various therapeutic preparations.

This paper is based partly on work reported to the U. S. Bureau of Chemistry, January 1, 1915, by Mr. W. D. Collins. Radioactive measurements made by the writer since that time have confirmed Mr. Collins' results and have also established the value of certain other methods. This brief account of the experience of the laboratory is presented merely to bring the matter to the attention of the Association with the hope that eventually uniform procedures for the measurement of radioactivity may be adopted by the Association.

In connection with the routine work, various methods of measurement have been tried and different forms of apparatus used.

The fontactoscope, an apparatus which comprises a gold leaf system and a very large detachable discharging chamber to hold samples of water from which the emanation is separated by shaking, would seem to be unsuited for accurate laboratory measurements, but in the field it is considered serviceable by many[1]. The U. S. Bureau of Chemistry has not found field experiments necessary as yet.

The gamma ray electroscope, which is used in measuring quantities of radium as large as a milligram, has not been needed.

An alpha ray electroscope[2] used by this laboratory for measurement of the radioactivity of substances in solid form makes possible a very rapid and fairly accurate examination of certain drugs and minerals.

An active solid introduced into the discharging chamber gives off chiefly alpha particles which ionize the enclosed air, thus allowing the charge imposed on a gold leaf to escape rapidly. The rate of fall of the leaf is noted just as with an emanation electroscope.

The electroscopes most used in this laboratory are of the emanation type and resemble one made by Charles W. Cook (Manchester, England) after the pattern of Rutherford[3]. They were made according to specifications which allowed for some modifications of the original. The comparatively small number of samples to be examined made it unnecessary to use an interchangeable electroscope of the kind described by Lind[4]. Also, since in many samples no radioactivity could be detected, it seemed better to have the measuring apparatus in complete fixed units.

For making accurate measurements of low radioactivity the Water Laboratory has followed the generally used method, which is to separate the radium emanation from the substance to be examined and then, by means of an electroscope, to compare the effect of this with the effect of emanation from a known amount of radium.

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[1] Engler, Sieveking and Koenig. Chem. Ztg., 1914, **38**: 425–7, 446–50; Physik. Z., 1914, **15**: 441–7.

[2] Rutherford. Radioactive Transformations. 1906, p. 28.

[3] Rutherford. Radioactive Substances and Their Radiations. 1913, p. 90.

[4] J. Ind. Eng. Chem., 1915, **7**: 406–10.

If the sample to be tested is a mineral water or solution, but little treatment is needed before separating the emanation by boiling. Soluble solids are dissolved in water or acids. Insoluble substances are fused with mixed sodium and potassium carbonates. This fusion is treated with dilute sodium carbonate solution and the residue remaining after filtration is dissolved in acid. This acid solution and the alkaline extract, which is now acidified, are placed in separate flasks, boiled and sealed. After about 4 days, half the emanation has formed and the gases containing it are collected. Boltwood's apparatus[1] for separating and collecting the emanation has been found very satisfactory. If the solution is boiled for about 20 minutes, the emanation appears to be separated completely.

The Schlundt and Moore apparatus[2] for collecting emanation by boiling it out, does not work properly unless given very close attention. It is possible also to extract emanation from solutions by bubbling air through while heating[3] and this method is often more simple and convenient than others. The gases obtained by boiling the solution are kept for 10 minutes, to allow for the decay of any thorium emanation present, and then transferred through a calcium chlorid drying tube to the discharging chamber of an emanation electroscope. The rate of fall of the charged gold leaf is observed about 3 hours after this. The electroscope is calibrated by measuring the rate of discharge caused by a known amount of emanation formed in a standard radium solution. The solution of radium barium bromid in hydrochloric acid (1.10 sp. gr.) used in the U. S. Bureau of Chemistry was furnished by the U. S. Bureau of Standards.

The electroscopes have been calibrated also by the use of pitchblende, the uranium content of which was determined as described by Brearley[4]. A sample of the ore is dissolved in nitric acid and the emanation collected and passed into the electroscope by Boltwood's method. Correction is made for the emanation lost spontaneously by sealing up a quantity of the ore in a glass tube and at the end of a month transferring the collected emanation to an electroscope by means of a stream of air. The temperature at which the sample is kept has an appreciable effect on the amount of emanation given off by the ore. If time permits, this correction may be eliminated by using the method of solution described by Lind and Whittemore[5] in which the ore is sealed up for a month in the flask where it is to be dissolved. Then, when the emanation in equilibrium with the ore is all confined, solution in acid gives the total emanation corresponding to the radium present.

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[1] Phil. Mag., 1905, 6th ser., **9**: 599–613; Am. J. Sci., 1904, 4th ser., **18**: 378–87.

[2] J. Phys. Chem. 1905, **9**: 320–32.

[3] Curie. Traité de Radioactivité. 1910, **1**: 284.

[4] Brearley. The Analytical Chemistry of Uranium. 1903; see also U. S. Bur. Mines Bull. 70, and J. Am. Chem. Soc., 1914, **36**: 2075–8.

[5] J. Am. Chem. Soc. 1914, **36**: 2071–2.

In many cases, to save time and manipulation, departure has been made from approved methods given in the literature but a detailed discussion is impossible here. For example, in most of the Water Laboratory's work the extra flask of Boltwood's collecting apparatus has been found unnecessary and a plain 100 cc. Erlenmeyer flask has been used for dissolving certain minerals and boiling strongly active solutions.

There has not been, as yet, occasion for making an extended investigation of thorium preparations. Thorium emanation has so short a life that it must be measured by passing it in a continuous stream through an electroscope. The method of Mache and Bamberger[1] seems to be the most satisfactory of any in the literature.

In dealing with mineral waters and most medicinal preparations this laboratory reports radioactivity in millimicrocuries (billionths (10^{-9}) of a curie)[2] per liter of solution or gram of substance. Many results in the literature have been expressed in mache or electrostatic units, but since so many investigators have not allowed for corrections (chiefly the Duane[3] correction) it is hard to place absolute reliance on their results or to make comparisons between results stated in the two different systems. For most purposes, however, the mache unit equals 0.37×10^{-9} curies or the electrostatic unit equals 0.37×10^{-6} curies is accepted as a satisfactory conversion ratio. The Water Laboratory accepts also the uranium-radium ratio of Heinmann and Marckwald[4]. (One gram of uranium equals 332.8×10^{-9} grams of radium.)

In regard to the magnitude of amounts of emanation determined, it may be said that the emanation electroscopes of the Water Laboratory have a limit of sensibility corresponding to 0.005×10^{-9} grams of radium, and will measure easily amounts of emanation corresponding to 20×10^{-9} grams of radium.

Some samples of artificial waters and medicinal preparations have been found to be as radioactive as claimed on the label. Most natural waters, when bottled, lose all their radium emanation after 30 days, because they do not contain dissolved radium salts or insoluble suspended radioactive matter to make them permanently active. Such waters, therefore, and also a number of artificial products, have been found so slightly radioactive at the time of sale that, in comparison with actual doses of radium salts used in medicine, and with the waters of natural springs known for their radioactivity at source, the radioactivity is wholly negligible.

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[1] Sitzb. kais. akad. Wiss. Wien. Abt., 1914, **123** (IIa): 334–45.

[2] One curie is the radioactivity corresponding to one gram of radium element.

[3] Compt. rend., 1905, **140**: 581–3; 1910, **150**: 1421–3.

[4] Physik. Z., 1913, **14**: 303–5.

WATER IN FOODS AND FEEDING STUFFS.

By W. J. McGee (Food and Drug Inspection Laboratory, U. S. Custom House, Savannah, Ga.), *Referee.*

The principal object of this year's work was the study of the method of drying without heat in a vacuum over sulphuric acid. The following outline of the method was sent out for trial:

DRYING IN VACUO WITHOUT HEAT.—OPTIONAL.

(This method was first suggested by Mr. Trowbridge at the meeting of the Association in 1909; approved in 1913 for final action as an optional official method in 1914; final action not yet taken.)

Mix the sample thoroughly and weigh about 2 grams by difference from a stoppered weighing bottle into tared crucibles provided with covers which are tared with crucibles. Place 200 cc. of fresh C. P. sulphuric acid in a good 6 inch vacuum desiccator. Put triplicate samples in the desiccator, smear the edges of the latter and the stop-cock with lubricant (a mixture of 3 parts of hard paraffin and 5 of vaseline) and exhaust by means of a vacuum pump. If a pump is not available, place 10 cc. of ether contained in a small beaker in the desiccator and exhaust with a water filter pump. It will be found convenient to interpose between the pump and the desiccator an empty bottle next to the desiccator and a bottle of water following this. Draw the air from the desiccator through the water and turn the desiccator stop-cock at just the instant when the water begins to rise in the tube leading from the empty bottle. Gently rotate the desiccator 4 or 5 times during the first 12 hours to mix the sulphuric acid with the water which has collected as an upper layer. At the end of 24 hours open the desiccator, forcing the incoming air to bubble through C. P. sulphuric acid. If a good vacuum has been maintained the samples are ready for the first weighing. After weighing place in a desiccator with fresh C. P. sulphuric acid and exhaust as before. Rotate the desiccator once or twice during the interval and weigh again at the end of 24 hours, repeating this process of drying in vacuo over sulphuric acid until the weight is constant.

REPORTS OF COLLABORATORS.

W. D. Richardson: Sausage meat (vacuum method).—Constant weight in about 208 hours with a loss in weight of about 1% less than by the heating methods.

D. B. Bisbee: Cheese (vacuum method).—Weight nearly constant eighth day. Loss of weight about 0.4% less than by heating methods.

J. O. Clarke: Cottonseed meal (vacuum method).—Weight practically constant in 9–15 days with loss about the same as in heating methods.

Corn meal.—In vacuum desiccator dried to approximately constant weight in 4 days and the loss was about 0.4% less than by heating methods.

Tomato ketchup.—With water content of 83.11% as indicated by heating at 100°C. in water oven, lost 80.15% in vacuo over sulphuric acid in 5 hours, and 81.21% in 5 days. In another experiment lost 80.53% in 18 hours and 80.72% in 42 hours. Both of these experiments were terminated by accident before constant weight was attained.

Hamburger steak.—In vacuo over sulphuric acid lost 70.42% of its weight in 4 days; by heating methods, 71.09%.

Lean meat.—Ground fine lost 65.56% in 44 hours and 67.10% by heating methods.

Apple jelly.—Lost 21.66% in 48 hours and 27.45% in vacuum oven at 70°C.

Cattle Food Laboratory, U. S. Bureau of Chemistry: Comparison of this method with drying at 100°C. at the house vacuum (average 86.4) gave the following results:

Mixed alfalfa, molasses, corn feed at 100°C.—Average pressure 86.4 mm. Moisture, 3.60%.

At 27°C.—Average pressure 7.5 mm. Moisture 1.89%.

Bran at 100°C.—Average pressure 86.4 mm. Moisture 10.79%.

At 27°C.—Average pressure 7.5 mm. Moisture 10.43%.

Linseed meal at 100°C.—Average pressure 86.4 mm. Moisture 10.86%.

At 27°C.—Average pressure 7.5 mm. Moisture 10.71%.

TABLE 1.

Moisture determinations under varying conditions.

(J. H. Roop, Indiana.)

SUBSTANCE	DRIED 5 HOURS IN HYDROGEN AT 100° C.	DRIED 5 HOURS IN VACUO AT 75° C.	DRIED IN 6 INCH VACUUM DESICCATOR WITH SULPHURIC ACID AVERAGE
	Per cent loss	*Per cent loss*	*Per cent loss*
Cottonseed meal	8.75	8.10	7.55
Wheat bran	11.65	11.27	11.04
Corn germ meal	4.82	4.45	4.36
Linseed meal	9.97	9.57	9.15
Corn and oats	13.01	12.42	12.68
Distillers' grains	8.90	7.95	7.64

C. O. Swanson: This method has been in use at the Kansas Agricultural College for several years and is considered very satisfactory. For flour the drying is continued for from 3–5 days. Very moist samples are allowed to remain in the desiccator for 3 weeks. A shaking machine is employed to keep the acid stirred up.

COMMENTS BY THE REFEREE.

It has been found at the U. S. Food and Drug Inspection Laboratory at Savannah that the desiccator acid containing ether and water can be recovered by digesting for about 4 hours over a free flame in a Kjeldahl flask with a drop of mercury. A study of this method from the work of this and previous years seems to show that it can be relied upon when heating the sample is inadmissible; and that it is valuable also when subsequent fat extraction is contemplated and heating would tend to harden the sample too much. For work of this kind in which time is not a factor it may be very convenient to have an official method.

TABLE 2.

Comparison of dehydrating powers of reagents used in desiccators at room temperature of 20°–33°C. and at atmospheric pressure.

SUBSTANCE	TIME	SULPHURIC ACID	CALCIUM CARBID	SODIUM METAL	CALCIUM OXID	CALCIUM CHLORID	SODIUM HYDROXID
	Days	*Per cent loss*	*Per cent loss*	*Per cent loss*	*Per cent loss*	*Per cent loss*	*Per cent loss*
Corn meal[1]:	1	10.18	10.06	....	9.54	9.22	7.76
	2	11.13	10.85	....	10.69	9.84	8.72
	3	11.49	11.22	....	11.09	10.05	9.21
	4	11.60	11.29	....	11.03	10.02	9.06
	5	11.74	11.43	....	11.20	10.10	9.19
	6	11.82	11.54	....	11.22	10.14	9.28
	7	11.87	11.53	....	11.30	10.14	9.23
Lean meat, finely ground[2]:	1	43.70	36.20	45.23	....	28.77	....
	2	65.15	63.22	62.69	....	56.41	....
	3	66.04	65.50	63.53	....	64.08	....
	4	66.40	65.97	63.89	....	64.75	....
	5	66.56	66.10	63.98	....	64.90	....
	6	66.65	66.27	64.13	....	65.12	....
	7	66.78	66.38	64.23	....	65.25	....
	10	66.98	66.62	64.40	....	65.50	....
	Hours						
Hamburger steak[3]:	3	9.00	11.93	....	....	8.73	....
	Days						
	1	59.26	62.17	....	....	54.74	....
	2	69.50	69.40	....	....	69.15	....
	3	70.16	69.97	....	....	69.82	....
	4	70.47	70.24	....	....	70.14	....
Tomato paste[4]:	1	77.51	40.16	77.10	....	77.32	....
	2	77.67	76.40	77.35	....	77.54	....
	3	77.98	77.10	77.50	....	77.64	....
Glucose and apple jelly[5]:	1	18.91	19.59	17.84	....	16.43	....
	2	....	19.62	18.07	....	16.57	....
Pure apple jelly[6]:	1	19.29	20.44	....	18.90	....	....
	2	20.17	20.84	....	19.78	....	....
	3	.....	21.11	....	20.19	....	....

[1] Vacuum oven 12.60%; in hydrogen 13.51%.
[2] Loss at 100°C., 67.10%.
[3] Loss at 100°C., 71.09%.
[4] Vacuum oven at 70°C., 78.4%.
[5] Vacuum oven at 70°C., 23.83%.
[6] Vacuum oven at 70°C., 27.45%.

It appears from this work and from the experience of previous years, particularly as reported at the meeting in 1913, that calcium carbid is a desiccator reagent nearly as efficient as sulphuric acid and one that is portable. This reagent is good until a large percentage of the lumps have loosened up into powder.

It will be best, however, to continue using sulphuric acid, lime or calcium chlorid for precipitates of copper suboxid.

Leaving phosphorus pentoxid out of the question on account of its high cost and its rapid decrease in efficiency, it appears that the 3 best desiccator reagents are sulphuric acid, calcium carbid and calcium oxid, in the order named.

RECOMMENDATIONS.

It is recommended—

(1) That further work be done in comparing the drying of various food and feeding products over sulphuric acid, calcium carbid and calcium oxid, with any other reagents thought desirable, at atmospheric pressure and in a partial vacuum.

(2) That the method of drying without heat over sulphuric acid as outlined above be finally made an official optional method.

DISCUSSION.

P. F. Trowbridge: Mr. Grindley of the University of Illinois has used the method with good results—much better than we have been able to obtain at our Missouri Laboratory. Some materials present difficulties because they dry to a horn-like substance. The referee has not sufficiently emphasized the need for a good vacuum. Seven millimeters of mercury are not enough. A pump is necessary. With a pressure not exceeding 1 mm. it is possible to obtain from 2–10% more moisture from grain than by drying at 102°C.

REPORT ON FOODS AND FEEDING STUFFS.

By G. L. Bidwell (Bureau of Chemistry, Washington, D. C.), *Referee.*

The coöperative work this year was all in charge of associate referees.

It is recommended—

(1) That the method for the detection of oats bleached with sulphur dioxid[1], be made official.

(2) That the method for determining the acidity of corn[2], be made official.

(3) That the method for determining the acidity of corn be studied to see if it is applicable to other materials.

(4) That the effect of preliminary drying on the ether extract content of feeds be studied.

(5) That the use of toluol or other suitable substance as a preservative for feed samples be studied.

(6) That the method of Bryan, Given and Straughn[3] for the estimation of sugar in feeds be studied and made official.

FEED ADULTERATION.

By C. Cutler (Agricultural Experiment Station, La Fayette, Ind.), *Associate Referee.*

The work during the past year was confined to the following recommendation, approved at the 1914 meeting:

"(1) That samples be sent out for the quantitative determination of adulterants on amounts varying from 5–25 grams to determine the smallest amount necessary to work with to get concordant results."

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[1] U. S. Bur. Plant Industry Circ. 40.

[2] Ibid., Bull. 199; U. S. Dept. Agr. Bull. 102.

[3] U. S. Bur. Chem. Circ. 71.

The following instructions were sent with the samples to 9 prospective collaborators:

INSTRUCTIONS FOR COLLABORATIVE WORK.

Quarter the samples, discard alternate quarters until the residue weighs approximately 25 grams. Procure a 10 gram sample in the same manner. A hand lens may be used in picking out small pieces of adulterants. The use of 20, 30 and 40 mesh sieves aids the work.

Sample No. 1 (wheat bran and screenings).—Determine the total amount of foreign material in 10 and 25 gram portions and identify the same.

Sample No. 2 (linseed meal containing cottonseed meal and hulls).—Determine the total amount of cottonseed meal and hulls in 10 and 25 gram portions.

Sample No. 3 (scratch feed).—Determine the total amount of grit in 10 and 25 gram portions; also the total amount of weed seeds present, identifying the principal ones.

Sample No. 4 (scratch feed containing coarse grit).

Sample No. 5 (wheat bran containing screenings).

RESULTS OF COLLABORATIVE WORK.

Per cent of adulterants found.

ANALYST	FOREIGN MATERIAL IN SAMPLE NO. 1		COTTONSEED MEAL AND HULLS IN SAMPLE NO. 2		WEED SEEDS IN SAMPLE NO. 3		GRIT IN SAMPLE NO. 3		GRIT IN SAMPLE NO. 4			SCREENINGS IN SAMPLE NO. 5	
	10 gram sample	25 gram sample	10 gram sample	25 gram mple sa	10 gram sample	25 gram sample	10 gram sample	25 gram sample	10 gram sample	25 gram sample	50 gram sample	10 gram sample	25 gram sample
A. W. Clark and O. C. McCreary, Agricultural Experiment Station, Geneva, N. Y.	4.2	3.2	5.0	4.1	32.1[1]	32.9[1]	22.6	24.0	....	...	...	...	...
B. H. Silberberg, Bureau of Chemistry, Washington, D.C.	7.5	7.6	.....	.....	......	......	19.1 24.4	21.5	....	...	...	...	...
G. W. Hoover and F. G. Smith, Transportation Building, Chicago, Ill.	5.1	4.2	0.58[2]	0.45[2]	33.5[1]	33.6[1]	23.6	21.1	....	...	...	...	...
P. H. Smith and Geo. H. Chapman, Agricultural Experiment Station, Amherst, Mass.	2.1	2.5	.....	.....	15.4[3]	14.0[3]	21.9	23.1	....	...	...	...	...
Carleton Cutler, Agricultural Experiment Station, La Fayette, Ind.	5.8	5.7	6.8	6.6	25.9	24.1	24.3 22.8	23.0 22.4	5.0	7.4	6.0 6.4	2.7	2.7

---

[1] Includes millet.

[2] Cottonseed hulls. Estimation of cottonseed meal present based on microscopical examination, 3-5%.

[3] Includes timothy, but not shrivelled alfalfa and clover seeds.

The greatest variation in these results is in the determination of grit in Sample No. 3. However, the average of the 10 gram samples compares favorably with the average of the 25 gram samples.

Sample No. 4, scratch feed, contained very coarse limestone grit. Determinations were made on carefully quartered 25 and 50 gram samples. The 2 determinations on the 50 gram sample gave 6.0 and 6.4% grit, showing a variation of only 0.4%; while the 10 gram sample gave 5%, and the 25 gram sample 7.4%. Thus, the larger sample gives more concordant results on feeds containing coarse grit.

The variation in percentage of weed seeds found by different analysts in scratch feed No. 3, is due to the fact that all small seeds, including millet, were determined by 2 of the collaborators reporting. The determinations on the 10 and 25 gram samples agree very closely, the average variation on 3 determinations being only 0.3%.

The determinations of screenings in Sample No. 1, and cottonseed meal and hulls in Sample No. 2, are fairly concordant on 10 gram and 25 gram samples.

The results on Sample No. 5, wheat bran and screenings, made on 10 and 25 gram samples show identical percentages of foreign materials.

RECOMMENDATIONS.

Based upon the facts brought out in this year's results, together with experience obtained during the past several years, it is recommended—

(1) That the following method for the approximate estimation of foreign material, excluding grit, in feeding stuffs, be adopted as a provisional method:

PREPARATION OF SAMPLE.

Thoroughly mix the original unground sample, quarter, discard diagonally opposite quarters until the remaining 2 quarters weigh 10 grams.

DETERMINATION.

Separate the 10 gram sample by means of 20, 30, 40 and 50 mesh sieves. With the aid of a hand lens, or other magnifying instrument, pick out foreign materials; first from the finest portion, and then from the next finest, and so on in order until all portions have been examined. Combine the foreign materials separated and weigh.

(2) That the size of sample of scratch and poultry feeds necessary to get concordant results on quantitative grit determinations be further investigated.

(3) That the following recommendations of 1914 be studied during the coming year:

"3. That methods for the detection of peat dried at high temperatures in feed stuffs be investigated," and "4. That the maximum percentage of foreign materials permissible in mill by-products be investigated".

REPORT ON CRUDE FIBER.

By C. K. Francis (Agricultural Experiment Station, Stillwater, Okla.), *Associate Referee.*

The work on crude fiber has been confined to a study of the official method, with linen and asbestos filters, and the one filtration method, using paper as a filter.

Samples of fat-free kafir corn and cottonseed meal were sent to the coöperating chemists with the following instructions:

INSTRUCTIONS TO COLLABORATORS.

Pass the sample through a 20 mesh sieve. Make all determinations in triplicate and report the time required for the analysis under each method.

Weigh portions of 2 grams on hard filter papers and extract 4 times with ether, or use the residue from the crude fat determination.

DETERMINATION OF CRUDE FIBER.

Method 1.—Official[1].

Make the first filtration through linen and the second through asbestos. Report fineness of the linen in number of threads to the inch, counting both ways. Dry and incinerate as directed under Method 2. Make blank determinations to show any loss of weight of the asbestos.

Method 2.—One Filtration Through Paper.

Brush carefully the dry, fat-free residue into an 800 cc. lipless beaker. Add 200 cc. of boiling 1.25% sulphuric acid and boil for 30 minutes, using round-bottomed flasks filled with cold water as condensers placed on top of the beakers. The flasks should fit closely if the beakers are round and have well-formed rims. (Kjeldahl flasks are used in the associate referee's laboratory.) Direct a gentle blast of air into the beaker when the foaming becomes serious. At the end of 30 minutes add 200 cc. of boiling 3.52% sodium hydroxid and continue the boiling for another 30 minutes.

A Büchner funnel, outside diameter 10.5 cm., is prepared with a 9 cm. S. & S. No. 575 hard filter paper. The paper should be moistened with water and pressed well into place, so that there will be no holes uncovered and no open channels left about the edges. It is advisable to place a glass filter plate over the paper, to protect it and to prevent excessive packing of the fiber.

After the 30 minutes alkali boiling, filter rapidly with the aid of suction, wash with boiling water, then with a 1.25% solution of concentrated hydrochloric acid (14 cc. made up to 500 cc. with water), until the washings are acid. Much time may be saved if the liquid is set aside for about 2 minutes to allow the fine material to settle so that the clear liquid may be decanted. Wash with hot water until free from chlorids, and finally wash the filter several times with 80% alcohol. Remove the paper from the funnel. Transfer the residue to a Gooch crucible with the aid of a spatula and a small amount of 80% alcohol. Rub the paper lightly with the finger to detach the last traces of the fiber residue.

Place the crucible in an oven heated to 105°–110°C. for from 2–6 hours, cool and weigh. The material may become dry in 2 hours but when large quantities of fiber are present a longer heating will be necessary. Heat for 30 minutes before weighing the second time.

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[1] U. S. Bur. Chem. Bull. 107, Rev., p. 56.

Partially cover the crucible and incinerate at a low red heat for 15 minutes or until a white or light gray ash is obtained. Cool and weigh. The loss of weight is crude fiber.

RESULTS OF COLLABORATIVE WORK.

TABLE 1.

Determination of crude fiber and time required.

ANALYST	KAFIR CORN				COTTONSEED MEAL				NUMBER OF THREADS TO THE INCH OF FILTERING CLOTH
	Official Method		Proposed Method		Official Method		Proposed Method		
	per cent	*hours*	*per cent*	*hours*	*per cent*	*hours*	*per cent*	*hours*	
O. C. Smith, Agricultural Experiment Station, Stillwater, Okla.	1.73	3	1.77	2	10.38	3	11.23	2	60 × 57
	1.70	......	1.69	......	10.20	......	10.59	......	....
	1.70	......	1.64	......	10.44	......	10.67	......	....
Average........	1.71	......	1.70	......	10.34	......	10.63	......	....
D. G. Morgan, Agricultural Experiment Station, Stillwater, Okla.	1.81	3¼	1.62	3	10.32	3¼	11.65	3	60 × 57
	1.80	......	1.78	......	10.42	.....	10.80	......	....
	1.83	......	1.64	......	10.24	......	10.61	......	....
Average........	1.81	......	1.68	......	10.33	......	10.70	......	....
G. W. Roark, Agricultural Experiment Station, College Station, Tex.	1.80	......	1.63	......	10.11	......	10.81	6	42 × 42
	1.77	......	1.10	......	10.37	......	......	......	....
	1.73	......	1.56	......	10.25	......	10.44	......	....
Average........	1.76	......	1.43[1]	......	10.24	......	10.62	......	....
A. S. Wells, Dairy and Food Commission, Portland, Ore.	1.75	8	1.90	7	9.57	10	11.01	16	92 × 92
	1.80	......	1.89	......	9.60	......	......	......	....
	1.75	......	1.94	......	9.80	......	10.82	......	....
Average........	1.76	......	1.91	......	9.65[1]	......	10.91	......	....
P. F. Trowbridge, Agricultural Experiment Station, Columbia, Mo.	1.52	10	1.64	7	10.05	10	11.16	7	68 × 71
	1.53	......	1.85	......	10.14	......	11.01	......	....
	1.75	......	1.81	......	10.18	......	11.25	......	....
Average........	1.60	......	1.76	......	10.12	......	11.14	......	....
G. P. Walton, Bureau of Chemistry, Washington, D. C.	1.70	4¼	1.54	9	10.87	4¼	10.17	9	42 × 46
	1.70	......	1.50	......	10.41	......	9.41	......	....
	1.79	......	1.53	......	10.33	......	10.07	......	....
Average........	1.73	......	1.52	......	10.53	......	9.88[1]	......	....

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[1] Omitted from corrected general average.

TABLE 1.—*Continued.*

ANALYST	KAFIR CORN				COTTONSEED MEAL				NUMBER OF THREADS TO THE INCH OF FILTERING CLOTH
	Official Method		Proposed Method		Official Method		Proposed Method		
	per cent	*hours*	*per cent*	*hours*	*per cent*	*hours*	*per cent*	*hours*	
Jas. W. Kellogg, Department of Agriculture, Harrisburg, Pa.	1.62	......	3.06	......	9.59	......	14.07	......	75 × 75
	1.57	......	......	......	9.59	......	......	......	....
	1.60	......	......	......	9.63	......	12.65	......	....
Average........	1.59	......	3.06[1]	......	9.60[1]	......	13.36[1]	......	....
W. D. Richardson, Swift and Company, Chicago, Ill.	1.47	3	1.55	2	9.13	4	10.03	4–5	87 × 87
	1.60	......	1.58	......	9.48	......	10.37	......	....
	1.56	......	1.40	......	9.78	......	10.55	......	....
Average........	1.54	......	1.51	......	9.46[1]	......	10.31	......	....
F. G. Smith, Transportation Building, Chicago, Ill.	1.64	1½	1.91	1¼	10.64	1½	11.19	4–8	58 × 54
	1.70	......	1.93	......	10.75	......	11.30	......	....
	1.74	......	1.96	......	11.01	......	11.99	......	....
Average........	1.69	......	1.93	......	10.80	......	11.24[1]	......	....
D. T. Evans, Fort Worth, Tex.	1.69	1	......	1⅓	10.10	1⅓	......	2	80 × 100
	......	......	......	......	9.95	......	......	......	....
	1.67	......	......	......	9.42	......	......	......	....
Average........	1.68	......	......	......	9.82[1]	......	......	......	....
J. H. Roop, Agricultural Experiment Station, La Fayette, Ind.	1.65	3	1.82	5	9.87	3	10.32	5	75 × 76
	1.63	......	1.82	......	9.81	......	9.92	......	....
	1.60	......	1.75	......	9.96	......	10.30	......	....
Average........	1.63	......	1.80	......	9.88[1]	......	10.18[1]	......	....
General average...	1.68	......	1.83	......	10.07	......	10.90	......	....
Corrected general average.	1.68	......	1.72	......	10.39	.....	10.72	......	....

[1] Omitted from corrected general average.

COMMENTS OF ANALYSTS.

G. W. Roark: Method 2 was shorter and easier than the regular (Official) method but there was difficulty in filtering through paper; also, it is believed, there is some loss around the edges of the paper.

A. S. Wells: Fine material passes through the linen during the first filtering. This is the main objection to the official method. The proposed method worked fairly well with the sample of kafir corn, but the sample of cottonseed meal packed so hard that it could not be removed without taking off some of the filter paper.

P. F. Trowbridge: After trying a great number of samples of muslin and linen we secured a very fine grade of muslin, 98 x 102 threads per inch. The best grade of linen we were able to secure ran 68 x 71 threads to the inch. Furthermore, the particles of fiber adhere much more to the linen threads than to the muslin threads.

Considerable difficulty was experienced in filtering through asbestos. The proposed method was more rapid than the official method, but the filtering was slow and there was difficulty in transferring the fiber from the paper to the Gooch crucible. Whenever any modification of the Sweeney method has been tried with single filtration higher results have always been obtained.

G. P. Walton: As pointed out in last year's report by this laboratory on crude fiber, this one filtration method so modifies the regular method as to make it extremely improbable that results from the 2 methods can be reconciled. Besides the question of the redissolving of substances precipitated, on making the acid liquid alkaline, there is the real objection to digesting with twice the volume of 1.25% sodium hydroxid solution specified in the official method.

The transfer of the fiber residuum is a tedious operation, and difficulty was experienced in obtaining all of it without including fiber from the filter paper.

Directions to wash finally with strong alcohol should be incorporated in the official method.

J. W. Kellogg: Proposed method entirely unsatisfactory when compared with the official method.

W. D. Richardson: With such samples as cottonseed meal it was found quite difficult to pass 400 cc. through the same filter, as the pores soon became clogged and made the filtration and washing unsatisfactory. Kafir corn gives good results by this method and is easy to handle.

F. G. Smith: Method 2 required from 4–8 hours to make the digestion and filtration, in one case it being practically impossible to complete the filtering.

D. T. Evans: The filtration through paper was so difficult that asbestos was substituted.

J. H. Roop: Great difficulty was encountered in filtering the alkaline solution through the paper and in transferring the fiber from the filter paper without including some fiber of the filter paper.

EXPERIMENTS BY THE ASSOCIATE REFEREE.

It may be of interest to mention some additional work on crude fiber. The data presented in Table 2 show the results obtained with 3 kinds of filters when but one filtration was made.

TABLE 2.

Determination of crude fiber in cottonseed meal by the one filtration method using different filters.

FILTERS	SEPARATE DETERMINATIONS			AVERAGE
	per cent	*per cent*	*per cent*	*per cent*
Linen	8.57	8.58	8.54	8.56
Hard paper	9.54	9.53	9.37	9.48
Asbestos	10.87	10.81	....	10.84

Portions of cottonseed meal from the same sample were examined in a similar manner, observing the directions of the official method.

TABLE 3.

Determination of crude fiber in cottonseed meal by the official method using different filters.

FILTER USED		SEPARATE DETERMINATION			AVERAGE	REMARKS
After acid digestion	After alkali digestion					
		per cent	*per cent*	*per cent*	*per cent*	
Linen	Linen	8.44	8.16	7.96	8.19	No suction; residue washed into a Gooch crucible.
Linen	Asbestos	8.56	8.82	......	8.69	Asbestos filter into a Gooch crucible, i.e., small amount asbestos used.
Linen	Asbestos	8.96	8.79	9.02	8.92	Asbestos filter in a Hirsch funnel, i.e., large amount asbestos.
Paper	Paper	9.63	9.53	9.51	9.56	S. & S. No. 575 paper in a Büchner funnel.

It will be observed from the data in Tables 2 and 3 that the determinations with a given filter, when considered alone, appear satisfactory, but when comparisons of the groups are made, the results do not agree.

Determinations in addition to those here reported have shown that the loss with linen depends upon the number of times the mixture is filtered through it, thus demonstrating that there is a loss of crude fiber.

The crude fiber determinations in which filter papers were used appear to be the most uniform for both methods. The paper filters certainly do not permit any solid matter to pass into the filtrate, and they offer a uniform filter which may be easily obtained and from which the residue can be readily transferred.

Some determinations were made in which asbestos was used for both filtrations according to the official method, but the large quantity of material added to the second filter, in this way, caused the filtration to proceed very slowly.

Portions of filter paper, S. & S. No. 597, were ground so as to pass a 40 mesh sieve. This material was intended to be used for testing the efficiency of different filters, but the residue obtained, after passing through the entire official method for crude fiber, showed so great a loss, that it became necessary to ascertain where the loss occurred. The final procedure involved digestion of separate samples in 1.25% sulphuric acid and in 1.25% sodium hydroxid, and in both reagents as outlined in the method for one filtration.

The results of this test are presented in Table 4.

TABLE 4.

Action of crude fiber reagents on pure filter paper.

PROCEDURE	FIBER RECOVERED BY USE OF PAPER FILTERS		FIBER RECOVERED BY USE OF ASBESTOS FILTERS	
	Original basis	Ash plus water-free basis	Original basis	Ash plus water-free basis
	per cent	*per cent*	*per cent*	*per cent*
Acid digestion only:	93.90 95.03 94.56	99.42 100.62 100.11	101.15 102.53 97.38	107.14 108.56 103.10
Average	94.49	100.05	100.35	106.27
Alkali digestion only:	91.33 92.27 91.78	96.70 97.72 97.17	98.06 98.52 98.74	103.86 104.38 104.59
Average	91.79	97.20	98.44	104.28
Acid and alkali, 1 filtration:	64.91 64.67	68.75 68.47	71.50 74.71 73.00	75.71 79.13 77.30
Average	64.79	68.61	73.07	77.38
Acid and alkali, 2 filtrations:	65.02 65.58 64.26	68.85 69.18 68.05		
Average	64.95	68.69	....	....

The action of the acid is apparently negligible, and the alkali does not seem to dissolve a very large quantity. However, the material digested in both acid and alkali showed a very large loss, approximately 31% by both methods. The asbestos filter gave higher results in all tests.

CONCLUSIONS.

The results reported this year indicate that hard filter paper, used in the manner suggested, is not satisfactory. All analysts obtained good results for kafir corn by the official method and the returns on the same sample tested by the one filtration method were encouraging. However, the showing for the sample of cottonseed meal was not so good and indicates a need for further study of crude fiber methods. A number of the chemists did not report the loss of weight due to the asbestos, but those reporting indicated that an error of from 0.05–0.20% plus, may be introduced. Results showing much higher losses were reported by several chemists.

THE DETERMINATION OF CRUDE FIBER[1].

By C. E. Mangels and P. F. Trowbridge (Agricultural Experiment Station, Columbia, Mo.).

In laboratories where a great many crude fiber determinations are made, it is important that the method used be as free as possible from tedious manipulations. As in all empirical determinations, however, it is important that the methods followed should be the same as in other laboratories until it has been shown that slight modifications do not affect the accuracy of the results.

In the collaborative work, the referee's samples were tested by methods which have been used in the Missouri Agricultural Experiment Station. The method in use this year and which seems to give the most satisfactory results is as follows:

> Transfer the residue from the ether extraction to a lipless beaker, add 200 cc. of cold 1.25% sulphuric acid and make a mark on the beaker to indicate the height of the liquid. Heat rapidly to boiling the contents of the beaker and continue the boiling for exactly 30 minutes. Filter rapidly the contents of the beaker through a muslin filter and wash the fiber free from acid with hot water. Place the original beaker under a funnel and transfer the fiber from the muslin to the beaker, using a spatula and a stream of 1.25% sodium hydroxid from a wash bottle. Then fill the beaker to the mark with 1.25% sodium hydroxid, heat rapidly to boiling the contents of the beaker and boil for exactly 30 minutes. Filter the hot alkaline solution through the same muslin filter, wash the beaker twice with hot water and transfer the washings to the filter. Then wash the fiber on the filter once with 1.25% hydrochloric acid and then thoroughly with hot water to remove all acid and chlorids. Transfer the bulk of the fiber from the muslin to a prepared Gooch crucible, spreading the fiber on the sides of the crucible so as to avoid clogging the filtration. Place the original beaker under the funnel and wash the last traces of the fiber from the muslin into the beaker by means of a stream of hot water. Connect the Gooch crucible with suction and transfer the fiber in the beaker to that in the crucible, by the aid of hot water and a policeman. Wash finally the fiber in the Gooch crucible with alcohol and dry to constant weight at 102°C., then ignite and weigh again to determine the fiber by difference.

The writers have tested a great many samples of linen and muslin to secure a satisfactory medium for filtering and have finally selected and secured a quantity, sufficient for several years, of close-woven muslin having 98 x 102 threads to the inch. The best linen which could be secured had 68 x 71 threads to the inch.

The method just described differs from the regular official method in that both the acid and alkali solutions are added to the sample cold, thereby making the contact with the sample 30 minutes in addition to the time required to heat the 200 cc. of solution to the boiling point. This, if anything, should give lower results than by the regular official method.

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[1] Presented by P. F. Trowbridge.

The results are shown under Method C in the following table and are slightly higher than results obtained by the regular official method (A). The writers have at different times tried the Sweeney method (single filtration) with various modifications, hoping to get a rapid method which was at the same time comparable with the regular official method.

In this method, the results of which are reported under (D) in the following table, 200 cc. of boiling 1.25% sulphuric acid were added to the fiber and the boiling continued for exactly 30 minutes. Two hundred cc. of boiling 3.52% sodium hydroxid were added immediately to this solution and the boiling continued for another 30 minutes. Rapid filtration was then made through the muslin filter. The beaker was washed twice with hot water, the washings being transferred to the muslin filter. The fiber was then washed once on the filter with cold 1.25% hydrochloric acid, and the washing completed with hot water. The transfer of the fiber and the completion of the determination were conducted exactly as described in the previous method. In length of time required this method is found to be only slightly shorter than the double filtration through muslin. The results obtained are seen to be higher in each case than those obtained by any of the other methods.

The results obtained by Mr. Francis' single filtration method through hardened filter are higher than either of the double filtration methods. For example, one of the single filtration methods gives 1.640%, on Sample No. 1, which is lower than any of the results obtained on this sample with our double filtration method and also lower than one of the results by the regular official method filtering once through linen and once through asbestos. On Sample No. 2 one of the determinations, 11.010, is lower than either of the results given by our double filtration method. These results would seem to indicate that the longer heating, caused by starting with the cold acid and alkali solutions, does not reduce the amount of crude fiber. The other modification in the method, by washing once with 1.25% hydrochloric acid, greatly facilitates the removal of the alkali and the chlorids and since it does not appear to affect the final amount of crude fiber its use is to be recommended.

Per cent of crude fiber obtained.

PROCEDURE	SAMPLE 1	SAMPLE 2	REMARKS
(A) Official method, first filtration through linen and second filtration through asbestos:	1.520 1.525 1.750	10.045 10.140 10.180	8-10 hours, filtration difficult.
Average	1.598	10.122	

Per cent of crude fiber obtained.—Continued.

PROCEDURE	SAMPLE 1	SAMPLE 2	REMARKS
(B) Smith-Francis method of filtration through hardened filter on Büchner funnel:	1.640 1.845 1.805	11.160 11.010 11.245	6–7 hours, filtration and transfer difficult.
Average	1.763	11.138	
(C) Missouri Station method, 2 filtrations through muslin, starting with cold 1.25% acid and alkali:	1.715 1.780 1.740	Lost 11.105 11.060	About 4½ hours, muslin used 98 x 102 threads per inch.
Average	1.745	11.083	
(D) Sweeney method, one filtration through muslin:	1.845 1.845 1.840	11.475 11.215 11.560	3½–4 hours.
Average	1.843	11.417	

REPORT ON SUGAR.

By C. A. Browne (Sugar Trade Laboratory, New York, N. Y.),
Referee.

Since the report of Mr. Cross in 1913, the work of the Association upon sugar has been interrupted, no referee having been appointed for the year 1913–1914.

In resuming the work which was so efficiently carried out by his predecessor, it occurred to the present referee that this Association during the next few years might in a measure retrieve a part of the damage which has resulted to the progress of sugar analysis as a consequence of the European war. Two international congresses had been scheduled this year to report upon sugar, that of the International Congress of Applied Chemistry, which was to meet in Petrograd in August, and that of the International Commission upon Uniform Methods of Sugar Analysis, which was to meet in Amsterdam in September. Both of these meetings have been indefinitely postponed and many years must elapse before the important questions which they were to consider are reported.

At the last meeting of the International Commission upon Uniform Methods of Sugar Analysis in New York in 1912, a committee was appointed to study the inversion constant of the Clerget method. As the present referee, in connection with his duties upon this committee, is conducting a series of researches along this line, he has selected the present occasion as an opportune one in which to report upon certain modifications of the Clerget method.

A STUDY OF CERTAIN MODIFICATIONS OF THE CLERGET METHOD

The volume to which the solution is made up for invert polarization which was formerly used by this Association and which is still employed by many sugar chemists, is the one originally proposed by Clerget. In this procedure 50 cc. of the clarified solution used for direct polarization were transferred to a flask graduated at 50 cc. and 55 cc. Concentrated hydrochloric acid was then added to the 55 cc. mark, the solution was mixed, a thermometer was inserted and the inversion was carried out according to the regulations prescribed for temperature and time of heating. After cooling, the solution was read upon the polariscope in a 200 mm. tube and the observed reading was increased by one-tenth to correct for the dilution from 50–55 cc. This corrected reading and the temperature of the solution were then substituted in the Clerget formula for calculating the percentage of sucrose.

One great advantage of diluting the solution from 50–55 cc. is that any errors in reading the inverted solution are increased by only one-tenth, whereas in the modifications which dilute from 50–100 cc. these errors are multiplied by 2. But notwithstanding this advantage, the experience of chemists has shown a certain unreliableness to exist in the old Clerget procedure so that in later years this method of making up to volume has been largely discontinued.

The referee believes that one great cause of the difficulty, which has been experienced with the old Clerget procedure, has been the neglect of a very serious error, viz: the diminution in volume which takes place in the 55 cc. of solution during inversion. This diminution of volume is due to three causes:

(1) The contraction in volume which all sucrose solutions undergo during inversion and which for 13 grams of sucrose in 55 cc. is about one-fourth of a cubic centimeter.

(2) The elevation in temperature produced by the addition of the hydrochloric acid. This elevation, for 5 cc. of concentrated hydrochloric acid to 50 cc. of sugar solution, is about 3°C., the cooling of the solution from 23°C. at the beginning to 20°C. at the end of the inversion produces a further slight contraction.

(3) The evaporation of water from the neck of the flask during inversion, the amount of such evaporation depending upon the diameter of the neck of the flask, and the time and temperature of inversion.

The combined influence of these 3 factors causes the volume of the 55 cc. of solution at the end of inversion to be about one-third of a cubic centimeter too small for the half normal weight of 13 grams.

It is essential to the accuracy of any method of double polarization that the volume of the solution after inversion be fixed with the utmost accuracy. The sources of error just mentioned are easily overcome if the volume of the inverted solution be brought to 55 cc. at the end of inversion and not at the beginning. If this precaution be followed more accurate results can be secured by the process of making the inverted solution up to 55 cc. than by any of the modifications which dilute to 100 cc. The control of temperature by this procedure is easily carried out by means of a thermometer placed in a control flask containing 55 cc. of blank solution. There is no danger of irregular mixing of the acid with the sugar solution, as the diffusion of the hydrochloric acid through the body of the liquid takes place quickly without shaking.

In employing this method the referee makes all his inversions at room temperature; 5 cc. of concentrated hydrochloric acid were added to the 50 cc. of sugar solution in a 50–55 cc. flask and after standing overnight the volume was completed to exactly 55 cc. at 20°C., after gently tapping the walls of the flask to detach any air bubbles which might have accumulated. The solution is then mixed and read in a polariscope which is also at a temperature of 20°C. The invert reading is then corrected by adding the necessary one-tenth.

The formula for calculating the sucrose by this method of inversion, when a normal weight of 26 grams of pure sucrose is taken, is

$$S = \frac{100\,(A - B)}{144.9 - \frac{t}{2}}$$

The above formula, corrected for the differences in specific rotation of invert sugar due to the varying concentration, when less than 26 grams of sucrose are dissolved to 100 cc., is

$$S = \frac{100\,(A - B)}{144.9 - \frac{t}{2} - 0.01\left[144.9 - \frac{t}{2} - (A - B)\right]} \text{ in which}$$

S = per cent of sucrose;
A = direct polarization;
B = corrected invert polarization;
t = temperature of the invert solution.

If the invert polarization be made at exactly 20°C. the above formula becomes

$$S = \frac{100\,(A - B)}{134.9 - 0.01\,[134.9 - (A - B)]}$$

The determinations of Table 1 give an idea of the comparative accuracy of this method when applied to solutions of pure sucrose.

TABLE 1.

Application of Clerget modified procedure to solutions of pure sucrose.

NUMBER	DIRECT POLARIZATION (A)	CORRECTED INVERT POLARIZATION (B)	TEMPERATURE (t)	SUCROSE (S)	
				Found	Taken
			°C.	*per cent*	*per cent*
1	99.90	−35.00	19.8	99.92	99.90
2	76.95	−26.79	19.6	76.97	76.92
3	57.70	−19.69	20.2	57.66	57.69
4	38.45	−13.04	20.2	38.44	38.46
5	19.20	− 6.49	20.0	19.15	19.23
6	7.70	− 2.53	20.0	7.65	7.69

TABLE 2.

Application of Clerget modified procedure to solutions of sucrose and dextrose.

NUMBER	DEXTROSE	DIRECT POLARIZATION (A)	CORRECTED INVERT POLARIZATION (B)	TEMPERATURE (t)	SUCROSE (S)	
					Found	Taken
	grams per 100 cc.			°C.	*per cent*	*per cent*
1	4.8	91.70	−11.88	20	76.96	76.92
2	4.8	72.45	− 5.06	20	57.70	57.69
3	4.8	53.15	+ 1.60	20	38.45	38.46
4	4.8	33.90	+ 8.20	20	19.23	19.23

The importance of using a formula, which corrects for the differences in specific rotation of invert sugar due to varying concentration, may be illustrated by taking the case of determination No. 3 in Table 2. This determination, using the uncorrected formula, gives 38.21 per cent sucrose, which is a quarter of a per cent too low.

In this connection the referee would very urgently recommend that the Association adopt a modified Herzfeld formula, or the Herzfeld table of factors, in place of the uncorrected formula[1]. The correction of the Herzfeld factor 142.66, for concentration has been done in several ways.

According to one method the corrected Herzfeld factor equals 141.78 + 0.0676 C, C being the grams of sucrose in 100 cc. The objection to this method is that a preliminary calculation with the uncorrected factor 142.66 is necessary in order to obtain an approximate value of C for determining the true factor. The work of calculation is thus doubled.

According to another method the corrected Herzfeld factor equals 141.84 + 0.05 n, in which n is the scale reading of the inverted solution. The objection to this second method is that it applies to inverted solutions of pure sucrose and is of no value when other optically active substances are present.

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[1] U. S. Bur. Chem. Bull. 107, Rev., p. 41.

The two objections named are overcome if the correction for concentration be based upon the difference between the direct and invert polarization. The Herzfeld formula as thus modified becomes

$$S = \frac{100\ (A - B)}{142.66 - \frac{t}{2} - 0.0065 \left[142.66 - \frac{t}{2} - (A - B)\right]}$$

which for 20°C. is simplified to

$$S = \frac{100\ (A - B)}{132.66 - 0.0065\ [132.66 - (A - B)]}$$

The above modified Herzfeld formula is the one which the referee would recommend to the Association for adoption.

A great deal of work has been done by different investigators during the past few years upon methods of double polarization in the effort to make them better adapted to the analysis of sugar mixtures. In the ordinary methods of procedure any levulose, or other sugar, whose optical activity is changed by the addition of the inverting acid, has a different rotation before and after inversion so that a considerable error may be introduced in the calculation of sucrose. The possible magnitude of this error may be seen from the determinations given in Table 3 which were made upon known mixtures of sucrose and invert sugar. The modified procedure of the original Clerget method was used.

TABLE 3.

Application of Clerget modified procedure to solutions of sucrose in invert sugar.

NUMBER	INVERT SUGAR	DIRECT POLARIZATION (A)	CORRECTED INVERT POLARIZATION (B)	TEMPERATURE (t)	SUCROSE (S)	
					Found	Taken
	grams per 100 cc.			*°C.*	*per cent*	*per cent*
1	1.10	94.60	−35.20	19.8	96.18	96.15
2	2.74	73.35	−30.42	20.0	77.10	76.92
3	5.48	50.70	−27.28	20.4	58.14	57.69
4	8.21	28.15	−24.04	20.4	38.99	38.46
5	9.86	6.90	−19.58	20.4	19.82	19.23

It is seen that as the amount of invert sugar increases there is a corresponding increase in the sucrose error. In case of honeys and other products containing a high percentage of levulose the error in the sucrose determination due to this cause may exceed 1%.

In mixtures of sucrose with invert sugar alone, it is possible to correct this error in the determination of sucrose by means of an empirical factor. Such a method of correction is not adapted, however, to the analysis of complex mixtures of optically active substances. The efforts of chemists have been directed, therefore, towards devising a method by which the direct and invert polarizations could be made under similar conditions.

Of the various methods which the referee has tested in this connection, the one which has given the most satisfactory results was that by means of invertase.

The use of invertase from yeast has long been recognized by chemists as the method of inversion which is most perfect in theory, but the difficulty of preparing satisfactory solutions of invertase has been a great obstacle against the introduction of the method. In 1909 Mr. Hudson devised several improvements for preparing invertase from yeast and in April 1910 published a paper upon the use of his invertase solution in the quantitative estimation of sucrose. In Hudson's method 50 cc. of the clarified solution used for the direct polarization are transferred to a 100 cc. flask, faintly acidified with acetic acid and then treated with 5 cc. of stock invertase solution. The volume is completed to 100 cc. and after inversion the solution read in the usual way, the invert reading being corrected for the optical activity of the invertase solution. The formula proposed by Mr. Hudson for this modification of the Clerget process is

$$\mathrm{S} = \frac{100\ (A - B)}{141.7 - \dfrac{t}{2}}$$

The assertion has been made that the influence of concentration upon the Clerget factor would practically disappear, provided the inverting agent was without influence upon the rotation of the invert sugar. This, however, is not the case and cannot be the case, since the specific rotation of invert sugar necessarily increases with the concentration. The referee has subjected the invertase method to a most careful examination and finds that the influence of concentration is just as pronounced as with the methods which employ hydrochloric acid.

The invertase solution employed by the referee was prepared according to the original procedure of Mr. Hudson and had the following properties:

Sp. gr. at $\frac{20^\circ\mathrm{C.}}{20^\circ}$ = 1.00363;
Refractive index = 1.33468;
Solids in 10 cc. solution = 0.1027 grams;
Ash in 10 cc. solution = 0.0040 grams;
Rotation of solution in 200 mm. tube = +0.65° Ventzke.

In using this invertase solution for the Clerget determination the referee followed the method proposed by Mr. Hudson except that the 50 cc. of sugar solution were treated with 10 cc. of invertase reagent instead of 5 cc. and the solution was warmed to 50°C., to hasten the inversion. The solution after standing overnight was made up to 100 cc. at 20°C. and polarized at this temperature. The invert polarization was then increased by 0.0065 to correct for the dextro-rotatory effect of the 10 cc. of invertase solution and then multiplied by 2 to correct for the dilution to 100 cc. The formula for calculating the percentage of sucrose by this method, as calculated from the average of many determinations upon sugar solutions of different concentration, was found to be

$$S = \frac{100\,(A - B)}{142 - \frac{t}{2} - 0.0065\left[142 - \frac{t}{2} - (A - B)\right]}$$

It will be noted that the concentration factor 0.0065 is the same as that found for the Herzfeld method, which follows the same method of diluting the 13 grams of inverted product to 100 cc.

The application of the above formula to the determination of sucrose in various mixtures with invert sugar is given in Table 4.

TABLE 4.

Application of invertase method to solutions of sucrose and invert sugar.

NUMBER	INVERT SUGAR	DIRECT POLARIZATION (A)	CORRECTED INVERT POLARIZATION (B)	TEMPERATURE (t)	SUCROSE (S)	
					Found	Taken
	grams per 100 cc.			*°C.*	*per cent*	*per cent*
1	0.00	99.80	−31.83	20	99.72	99.80
2	0.18	95.85	−30.83	20	95.99	95.96
3	0.43	89.75	−29.33	20	90.27	90.20
4	0.67	85.60	−28.23	20	86.31	86.37
5	0.92	79.50	−26.73	20	80.58	80.61
6	2.44	47.00	−18.63	20	49.89	49.90
7	2.44	27.90	−12.43	20	30.69	30.71

The results show that the invertase method gives far more accurate results than the method of acid inversion in the analysis of complex mixtures which contain sucrose and levulose.

The referee does not believe that the invertase method will immediately displace the method of acid inversion for purposes of commercial analysis. The preparation of the invertase solution is somewhat troublesome and the gradual deterioration of the reagent involves the risk of incomplete inversion. The process is one which requires care and watchfulness. A blank analysis upon a weighed amount of pure sucrose should be carried out with every set of determinations. The invertase method is invaluable, however, as a control upon the accuracy of the method of acid inversion and the referee would recommend that it be adopted as a provisional method by the Association.

RECOMMENDATIONS.

In planning the work for next year the referee believes that attention should be devoted to those matters which are of most pressing importance to sugar analysis. He would, therefore, recommend—

(1) That the study of the modifications of the Clerget method be continued with special reference to the accurate determination of sucrose in complex mixtures of carbohydrates.

(2) That efforts be made towards the adoption of an accurate method for determining small quantities of invert sugar in the presence of large amounts of sucrose. In this connection the colorimetric methods for estimating invert sugar should be examined.

(3) That next year's referee in collaboration with the U. S. Bureau of Chemistry establish a table of reduction factors for the more common reducing sugars.

There are a number of improvements and changes, which the progress of sugar analysis, during the past 10 years, requires to be made in the official and provisional methods of the Association. The referee has gone over these methods with several of the members of the Committee on Editing Methods of Analysis and it was considered advisable to present the following recommendations:

(4) That the official text of the methods of the International Commission be substituted for the present text[1] and that these methods of the International Commission be brought up to date.

(5) That the following method for preparing a salt-free alumina cream be adopted in place of the present method[2]:

Precipitate concentrated alum solution with a slight excess of ammonium hydroxid and wash the precipitate by decantation with water until the solution is free from sulphates. Pour off the excess of water and store the residual cream in a stoppered bottle.

(6) That at the end of section (c)[3] the following be inserted:

For concentrations of sucrose of less than 13 grams to 100 cc. of invert solution the following general formula should be used.

$$S = \frac{100\,(P - I)}{142.66 - \frac{t}{2} - 0.0065\left[142.66 - \frac{t}{2} - (P - I)\right]}$$

The above formula is applicable to the determination of sucrose in the presence of dextrose, commercial glucose, and all other substances whose optical activity is not affected by the inverting acid. With materials which contain much levulose, such as honey, fruit products, etc., the method gives too high results.

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[1] U. S. Bur. Chem. Bull. 107, Rev., pp. 39–40.

[2] Ibid., p. 40.

[3] Ibid., p. 41.

(7) That at the end of section (d)[1] the following formula be substituted for the present formula for calculating the per cent of sucrose in the presence of raffinose:

$$S = \frac{0.5124\,P - I}{0.839}$$

The above formula supposes that the polarizations be made at exactly 20°C. If the temperature (T) be other than 20°C., the following formula should be used:

$$S = \frac{P\,(0.4724 + 0.002\,T) - I}{0.899 - 0.003\,T}$$

Having calculated S,

$$R = \frac{P - S}{1.852}$$

(8) That after section (d)[1] the following be inserted:

DETERMINATION OF SUCROSE BY MEANS OF INVERTASE.—PROVISIONAL.

Preparation of Invertase Solution, Hudson Method.

Break up 5 pounds of pressed yeast, which may be either baker's or brewer's yeast, add 30 cc. of chloroform to it in a closed flask and allow to stand at room temperature (20°C.) overnight. By morning the solid mass will have become fluid and it should then be filtered through filter paper allowing several hours for draining. To the filtrate add neutral lead acetate until no further precipitate forms and again filter. Precipitate the excess of lead from the filtrate with potassium oxalate and filter. To this filtrate add 25 cc. of toluene and dialyze the mixture in a pig's bladder for 2 or 3 days against clear running tap water. The dialyzed solution should be colorless, perfectly clear after filtration, and neutral to litmus; it should be preserved in an ice-box with the addition of a little toluene to prevent the growth of micro-organisms. The optical activity of the invertase solution is noted and a correction for this, according to the amount of solution used, must be applied to the invert reading.

Determination.

Dissolve the normal weight (26 grams) of substance in water, clarify, make up to volume, and take the direct polarization (P) as under section (d). Remove the excess of lead from the filtrate, if lead has been used as a clarifying agent, with anhydrous sodium carbonate or potassium oxalate, and filter. To 50 cc. of the filtrate in a 100 cc. flask add acetic acid by drops until the reaction is acid to litmus, add 10 cc. of the stock invertase solution and let stand in a warm place (about 40°C.) overnight. Cool and make up to 100 cc. at 20°C. Polarize at 20°C. in a 200 mm. tube. Allow the solution to remain in the tube for an hour and repeat the polarization. If there is no change from the previous reading the inversion is complete, when the reading and temperature of the solution are carefully noted. The reading is corrected for the optical activity of the invertase solution and then multiplied by 2. The percentage of sucrose is then calculated by the following formula:

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[1] U. S. Bur. Chem. Bull. 107, Rev., p. 41.

$$S = \frac{100\ (P - I)}{142 - \frac{t}{2} - 0.0065 \left[142 - \frac{t}{2} - (P - I)\right]}$$

S = per cent of sucrose;
P = direct reading;
I = invert reading;
t = temperature at which invert reading is made.

(9) That before section (c)[1] the following be inserted:

Reducing sugars other than dextrose may be determined, using Allihn's modification of Fehling's solution, by means of the above table and method by use of the following factors:

Arabinose = Glucose × 0.969;
Xylose = Glucose × 1.017;
Levulose = Glucose × 1.093;
Invert sugar = Glucose × 1.044;
Galactose = Glucose × 1.114.

(10) That before section (3. Ash[2]) the table for specific gravity and total solids of sucrose solutions at $\frac{20^\circ\text{C.}}{4^\circ}$ used by the U. S. Bureau of Standards and Reichsanstalt of Germany be inserted.

(11) That in the Munson and Walker table[3] for calculating lactose the column for lactose with one-half molecule of water be omitted.

No report was made by the referee on the general subject of the separation of nitrogenous substances.

REPORT ON THE SEPARATION OF NITROGENOUS SUBSTANCES (MEAT PRODUCTS).

By P. F. Trowbridge (Agricultural Experiment Station, Columbia, Mo.), *Associate Referee.*

No collaborative work has been undertaken. In connection with other work, a number of carefully prepared samples have been secured upon which it is planned to study the products of dissociation.

It is recommended that the referee for next year attempt to determine the relative amounts of some of the dissociation products in water-soluble and water-insoluble meat proteins.

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[1] U. S. Bur. Chem. Bull. 107, Rev., p. 51.
[2] Ibid., p. 67.
[3] Ibid., pp. 243–51.

REPORT ON THE SEPARATION OF NITROGENOUS SUBSTANCES (MILK AND CHEESE).

By Leroy S. Palmer (Agricultural Experiment Station, Columbia, Mo.), *Associate Referee.*

The recommendation adopted for this work at the last meeting of the Association called for studies leading to the adoption of methods for the determination of the non-casein proteins and the products of protein decomposition in milk. Your referee begs to report that a serious illness last spring prevented his giving this work the attention originally planned. Some progress has been made, however, and although the results secured to date are too indefinite for detailed report, the work is being continued.

In the report made last year attention was called to the fact that the combined official method of the Association for casein, and the provisional method for the so-called albumin does not remove all the substances from milk that are of true protein character. This is particularly true in old milk that has undergone protein decomposition; but the writer wishes to point out that even the very freshest milk will usually show a non-casein-non-heat-coagulable-protein percentage approximately equal to the percentage of heat-coagulable protein obtained by the present provisional method. The following table shows the analyses of six 10 cc. portions of the same skim milk[1], the analyses being started about 3 hours after the milk was drawn.

Per cent of protein in milk by different methods.

METHOD	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4	SAMPLE 5	SAMPLE 6
Total protein (official)	3.80	3.80	3.80	3.80	3.80	3.80
Casein (official)	2.89	2.89	2.89	2.84	2.84	2.88
Heat-coagulable proteins (provisional)	0.526	0.502	0.451	0.529	0.544	0.510
Residual proteins (precipitated by Almén's reagent)	0.280	0.280	0.288	0.273	0.322	0.274
Residual nitrogen (as protein not precipitated by Almén's reagent)	0.160	0.205	0.301	0.232	Lost	0.205

Other figures could be presented showing relatively the same results for other samples of milk. The preceding figures show approximately 8% of the total true protein of fresh milk not recovered by acid precipitation or heat coagulation, a percentage sufficiently great to demand attention. Unless further study shows that these residual proteins, at least those precipitated by Almén's tannic acid reagent, are formed as the result of the present methods of analysis for casein and heat-coagulable protein, it would seem that the presence of these simpler protein substances in fresh milk should be recognized, and suitable methods incorporated in the official methods for their determination. Careful distinction should be made at the same time between these substances present in fresh milk, and the products of protein decomposition, some of which would be determined by the same method. The data at least serve to call attention to the need of a careful study of the origin and exact character of these protein bodies present in fresh milk, which would ordinarily be called proteoses and peptones.

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[1] Mixed milk from the University herd.

RECOMMENDATION.

It is recommended—

(1) That the studies be continued leading to the adoption of methods for the determination of the non-casein proteins and the products of protein decomposition in milk.

REPORT ON DAIRY PRODUCTS[1].

By Lewis I. Nurenberg (State Department of Health, Boston, Mass.)
Referee.

A study, and compilation of figures, has been made of the sour serum method for the detection of added water in milk. In this work the referee has had the coöperation of Messrs. J. T. Keister, associate referee, and L. W. Ferris, of the Bureau of Chemistry, Washington, D. C., and George B. Taylor, J. R. Keeney, and J. S. Slack, of the State Board of Health, New Orleans, La.

The referee has obtained figures of over 660 samples of known purity milk from individual cows, and 49 samples from herds. These samples are representative of practically all breeds of cows mentioned in the 1914 report and of such influencing conditions as variation in season and lactation period. More than one-half of the total number represent low-grade milk.

The methods of preparation and analysis of the serum are as follows:

PREPARATION OF THE SOUR SERUM[2].

Allow the milk to sour spontaneously, and filter.

REFRACTION OF SOUR SERUM.

Determine the index of refraction of the clear serum at 20°C. by means of the Zeiss immersion refractometer. A refraction below 38.3 indicates added water.

Transfer 25 cc. of the serum to a flat-bottomed platinum dish and evaporate to dryness over the water bath. Then heat the contents of the dish over the small flame (to avoid sputtering) until charred. Place the dish in an electric muffle, with pyrometer connected, and ash at a heat not greater than 500°C., or 900°F. Cool and weigh. Express result in grams per 100 cc. An ash below 0.730 indicates added water.

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[1] Presented by H. C. Lythgoe.

[2] Matthes and Müller. Z. offent. Chem., 1903, **9**: 173–8.

In the original article as described by A. Burr and F. M. Berberich[1], the authors have outlined the following method:

ASH OF SOUR SERUM[1].

Measure 50 cc. of the serum into a platinum dish, evaporate to dryness, and carbonize over a low flame. Extract the char with hot water, burn the insoluble residue, add the solution to this ash, evaporate to dryness, ignite at a low temperature and weigh.

Experience has shown, however, with the exercise of care and an accurate pyrometer that the former stated method is as accurate as the latter, and involves less manipulation.

Table 1 contains the refractions of the sour serum of 660 samples of known purity milk from individual cows and 49 samples from herds. In the case of the individual cows the variation in the readings extends from 38.3–45.9. The greatest percentage of samples (25.2%) refracts between 41 and 42. There appears to be a certain uniformity each side of this medial point. The percentage of samples refracting between 40 and 41, and 42 and 43 is practically the same (from 18–20%); between 39 and 40, and 43 and 44 the percentage is from 12–13.5%, while from 38.3–39 and 44–45 the percentage is 4–5%.

In the herds the largest percentage of samples (75%) refracts between 41 and 43. Between 40 and 41 and 43 and 44 the percentage is from 10–12. As would be expected, no samples were found refracting at the maximum (45.9) or minimum (38.3) limits.

TABLE 1.

Variation in refractive indices of sour serum of known purity milk.
(660 samples from individual cows; 49 samples from herds.)

INDIVIDUAL COWS			HERDS		
Reading at 20°C.	Number of samples examined	Per cent of samples examined	Reading at 20°C.	Number of samples examined	Per cent of samples examined
38.3–38.9	34	5.2	38.3–38.9	0	0
39–39.9	81	12.3	39–39.9	2	4.1
40–40.9	121	18.3	40–40.9	6	12.3
41–41.9	167	25.2	41–41.9	18	36.7
42–42.9	133	20.2	42–42.9	18	36.7
43–43.9	90	13.6	43–43.9	5	10.2
44–44.9	27	4.1	44–44.9	0	0
45–45.9	7	1.1	45–45.9	0	0

Following is the graphic representation of the figures given in Table 1. The curves cross at the 20% line with a refractive index of 40.8 and again at the 15% line with a refractive index of 43.3.

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[1] Chem. Ztg., 1908, **32**: 617–8.

VARIATION IN REFRACTIVE INDICES OF SOUR SERUM OF KNOWN PURITY MILK.

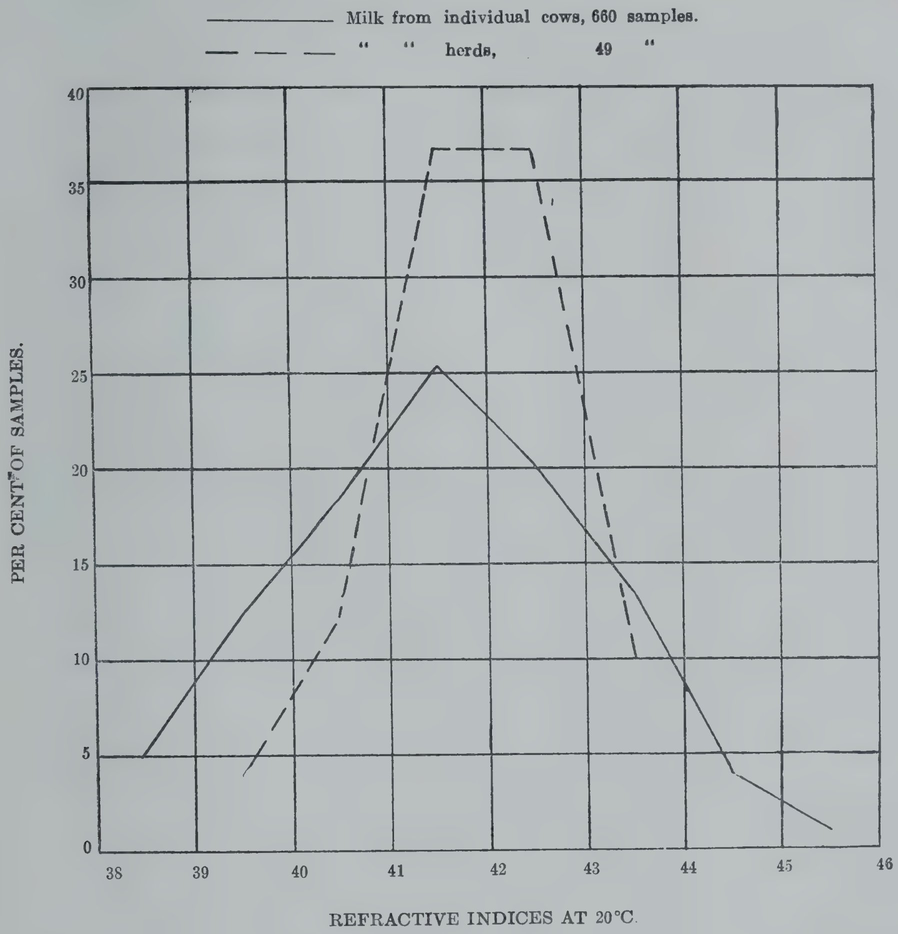


TABLE 2.

Variation in ash of sour serum of known purity milk.
(504 samples from individual cows; 35 samples from herds.)

INDIVIDUAL COWS			INDIVIDUAL COWS			HERDS		
Ash gram per 100 cc.	Number of samples examined	Per cent of samples examined	Ash gram per 100 cc.	Number of samples examined	Per cent of samples examined	Ash gram per 100 cc.	Number of samples examined	Per cent of samples examined
0.730–0.739	41	8.2	0.840–0.849	17	......	0.730–0.739	......	......
0.740–0.749	54	......	0.850–0.859	7	4.8	0.740–0.749	1	2.9
0.750–0.759	34	17.3						
			0.860–0.869	16	......	0.750–0.759	2	......
0.760–0.769	51	......	0.870–0.879	7	4.6	0.760–0.769	4	17.1
0.770–0.779	38	17.7						
			0.880–0.889	7	......	0.770–0.779	2	......
0.780–0.789	52	......	0.890–0.899	4	2.2	0.780–0.789	6	22.8
0.790–0.799	45	19.3						
			0.900–0.909	4	......	0.790–0.799	6	......
0.800–0.809	51	......	0.910–0.919	......	0.8	0.800–0.809	9	42.9
0.810–0.819	37	17.3						
			0.920–0.929	1	......	0.810–0.819	4	......
0.820–0.829	26	......	0.930–0.939	1	0.4	0.820–0.829	1	14.3
0.830–0.839	9	7.0						
			0.940–0.949	2	0.4	0.830–0.949	......	......

Table 2 presents the sour serum ash figures of 504 samples of known purity milk from individual cows and 35 samples from herds. In the individual cows only 8.2% of the ash figures extended between the minimum limit 0.730 and 0.740, while 71.6% were between 0.740 and 0.820; 16.4% of the figures were between 0.820–0.880, and 3.8% between 0.880–0.950. In the milk from herds no samples were found with ash figures from the minimum limit 0.730–0.739, or from 0.830 to the maximum limit 0.949; 2.9% of the ash figures were between 0.740–0.749; 82.8% were between 0.750 and 0.810; and 14.3% were between 0.810 and 0.830.

The data from Table 2 are shown in the following plot. The curves cross at the 17.5% line with an ash percentage of 0.761.

VARIATION IN ASH OF SOUR SERUM OF KNOWN PURITY MILK.

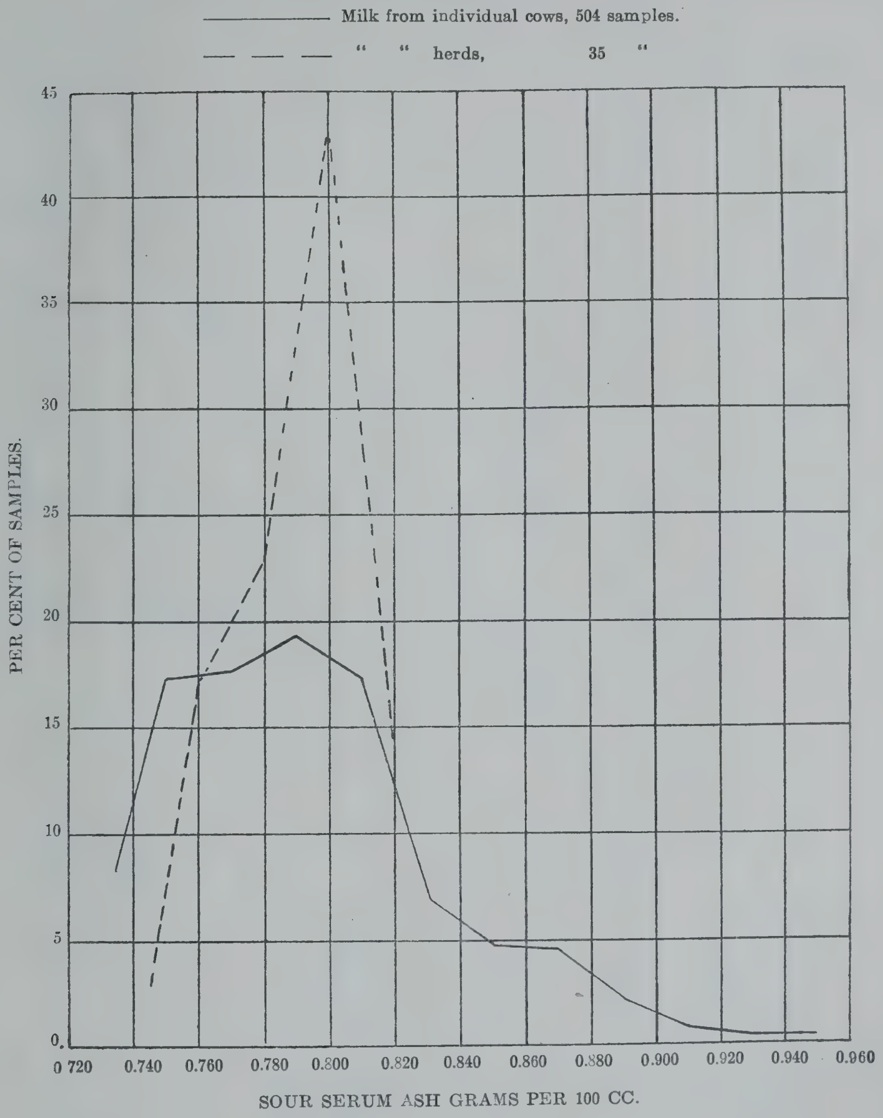


TABLE 3.

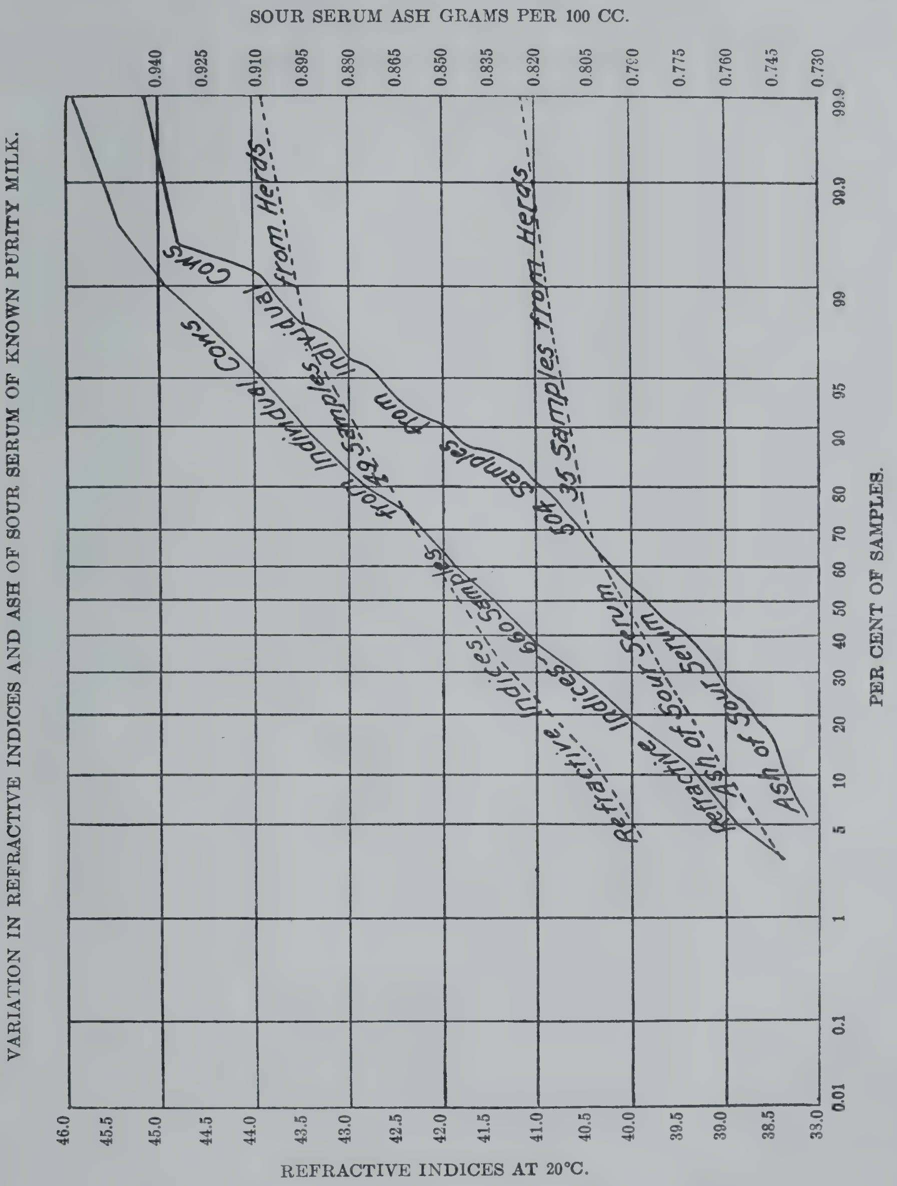
Variation in refractive indices and ash of sour serum of known purity milk.
From individual cows.

REFRACTIVE INDICES	NUMBER OF SAMPLES EXAMINED	PER CENT OF SAMPLES EXAMINED	ASH GRAM PER 100 CC.	NUMBER OF SAMPLES EXAMINED	PER CENT OF SAMPLES EXAMINED	ASH GRAM PER 100 CC.	NUMBER OF SAMPLES EXAMINED	PER CENT OF SAMPLES EXAMINED
38.0–38.4	20	3.0	0.730–0.734	30	5.9	0.840–0.844	8	1.6
38.5–38.9	14	2.1	0.735–0.739	11	2.2	0.845–0.849	9	1.8
39.0–39.4	45	6.8	0.740–0.744	33	6.5	0.850–0.854	3	0.6
39.5–39.9	36	5.5	0.745–0.749	21	4.1	0.855–0.859	4	0.8
40.0–40.4	60	9.1	0.750–0.754	19	3.8	0.860–0.864	8	1.6
40.5–40.9	61	9.2	0.755–0.759	15	3.0	0.865–0.869	8	1.6
41.0–41.4	87	13.2	0.760–0.764	33	6.5	0.870–0.874	5	1.0
41.5–41.9	80	12.1	0.765–0.769	18	3.6	0.875–0.879	2	0.4
42.0–42.4	87	13.2	0.770–0.774	26	5.2	0.880–0.884	4	0.8
42.5–42.9	46	7.0	0.775–0.779	12	2.4	0.885–0.889	3	0.6
43.0–43.4	52	7.9	0.780–0.784	31	6.1	0.890–0.894	1	0.2
43.5–43.9	38	5.8	0.785–0.789	21	4.1	0.895–0.899	3	0.6
44.0–44.4	18	2.7	0.790–0.794	25	4.9	0.900–0.904	3	0.6
44.5–44.9	9	1.4	0.795–0.799	20	4.0	0.905–0.909	1	0.2
45.0–45.4	5	0.7	0.800–0.804	32	6.3	0.910–0.914	......	......
45.5–45.9	2	0.3	0.805–0.809	19	3.8	0.915–0.919	......	......
Total.....	660	100.0	0.810–0.814	27	5.4	0.920–0.924	1	0.2
			0.815–0.819	10	2.0	0.925–0.929	......	......
			0.820–0.824	20	4.0	0.930–0.934	1	0.2
			0.825–0.829	6	1.2	0.935–0.939	......	......
			0.830–0.834	7	1.4	0.940–0.944	2	0.4
			0.835–0.839	2	0.4			
						Total.......	504	100.0

From herds.

REFRACTIVE INDICES	NUMBER OF SAMPLES EXAMINED	PER CENT OF SAMPLES EXAMINED	ASH GRAM PER 100 CC.	NUMBER OF SAMPLES EXAMINED	PER CENT OF SAMPLES EXAMINED
38.0–38.4	....	....	0.730–0.734	....	....
38.5–38.9	....	....	0.735–0.739	....	....
39.0–39.4	....	....	0.740–0.744	1	2.9
39.5–39.9	2	4.1	0.750–0.754	2	5.7
40.0–40.4	4	8.2	0.760–0.764	2	5.7
40.5–40.9	2	4.1	0.765–0.769	2	5.7
41.0–41.4	8	16.3	0.770–0.774	1	2.9
41.5–41.9	10	20.4	0.775–0.779	1	2.9
42.0–42.4	11	22.4	0.780–0.784	2	5.7
42.5–42.9	7	14.3	0.785–0.789	4	11.4
43.0–43.4	4	8.2	0.790–0.794	4	11.4
43.5–43.9	1	2.0	0.795–0.799	2	5.7
Total......	49	100.0			
			0.800–0.804	4	11.4
			0.805–0.809	5	14.3
			0.810–0.814	4	11.4
			0.820–0.824	1	2.9
			Total..........	35	100.0

Table 3 contains the refractive indices and sour serum ash figures arranged for the arithmetic probability curve, which follows.



If the samples were fairly representative, and if the variable follows the law of probability, the resulting curve will be a straight line. Although these lines are not straight, yet they are so nearly straight that it can be said that sufficiently representative samples have been taken to draw definite conclusions.

The medial line (50% line) shows that 50% of the samples had refractive indices below 41.4 from individual cows, and 41.8 from herds. The curves for the refractive indices of the herds and individual cows cross at the 73% point with refractive index of 42.4. The medial line also shows that in 50% of the samples the sour serum ash was below 0.784 from individual cows and 0.793 from herds. The curves for the sour serum ash of the herds and individual cows cross at the 68% line with the sour serum ash of 0.802.

Table 4 contains the results submitted by Mr. L. W. Ferris of the U. S. Bureau of Chemistry. His experimental work has shown conclusively that the time of spontaneous souring affects the sour serum ash. In the cases of partial souring the sour serum ash figure is lower than the acetic serum ash which, of course, should not be. Unless the sample is sufficiently sour, some of the calcium salt is precipitated with the casein and the sour serum ash is very liable to be low, as shown in Table 4. The minimum sour serum ash figure, reported as 0.671, on longer souring was raised to 0.722 which corresponds to the figure obtained by the acetic serum after being multiplied by 1.02.

COMMENTS BY ANALYSTS.

All samples except the first 8 represent single milkings from individual cows. The acetic acid serum was obtained by adding 2 cc. of 25% acetic acid to 100 cc. of milk. The results were multiplied by 1.02 to make them comparable with the results on the spontaneous serum. The results obtained by both methods are about the same. The degree of souring of the spontaneous serum appears to make no difference in either the ash or refraction, although clearer serums were obtained by allowing the sample to stand until the whey and curd had separated. In some cases it was impossible to get a clear serum. The spontaneous souring involves less work than the acetic acid method and in most cases the spontaneous serum is about as clear as that obtained by the acetic acid method.

One sample gave a refraction of 37.9 (cow No. 15) on the spontaneous serum and samples from 4 different cows (Nos. 2, 5, 6 and 7) gave an ash of serum below 0.73.

Table 5 contains the results submitted by Mr. J. T. Keister, associate referee. In this table there are 3 abnormal sour serum ash figures. The referee is of the opinion that in each case the milk was not thoroughly sour. If the sample is thoroughly sour, a clear serum will be obtained which will contain all the mineral matter.

TABLE 4.

Data for detection af added water to milk. (September–October, 1915.)
(Leslie W. Ferris, Washington, D. C.)

LABORATORY NUMBER	NUMBER OF COW	BREED	AGE OF COW	TIME SINCE CALVING	AMOUNT OF MILK	TIME OF MILKING	SPONTANEOUS SERUM			ACETIC ACID SERUM		
							Refraction at 20°C.	Ash	Lactic acid	Refraction at 20C.	Ash × 1.02	Lactic acid
			years	*months*	*pounds*		*reading*	*gram per 100 cc.*	*gram per 100 cc.*	*reading*	*gram per 100 cc.*	*gram per 100 cc.*
9420½		Market milk	......	....	....	....	41.6	0.798	....	....	....	....
9421		Market milk	......	....	....	....	42.0	0.766	0.472	42.3	0.788	0.727
9422		Market milk	......	....	....	....	41.9	0.773	0.365	42.3	0.796	0.691
		Same sample, longer souring	......	....	....	....	41.5	0.785	0.596	....	....	....
9423		Market milk	......	....	....	....	40.7	0.782	0.508	42.0	0.780	0.657
		Same sample, longer souring	......	....	....	....	41.1	0.781	0.601			
9423		Market milk	......	....	....	....	41.5	0.775	0.443	41.7	0.783	0.695
		Same sample, longer souring	......	....	....	....	40.7	0.776	0.508	....	....	....
9428	1	Holstein	$4\frac{2}{3}$	6	20	1 p.m.	39.7	0.802	0.639	40.2	0.804	0.634
		Same sample, longer souring	......	....	....	....	39.9	0.802	0.675	....	....	....
9434	1	Holstein	$4\frac{2}{3}$	6	20	1 p.m.	39.7	0.801	0.497	39.4	0.814	0.733
		Same sample, longer souring	......	....	....	....	39.9	0.802	0.675	....	....	....
		Same sample, longer souring	......	....	....	....	39.4	0.803	0.695	....	....	....
9436	1	Holstein	$4\frac{2}{3}$	6	12.5	7.30 p.m.	38.7	0.796	0.727	39.7	0.801	0.790
		Same sample, longer souring	......	....	....	....	39.1	0.811	0.713	....	....	....
9438	1	Holstein	$4\frac{2}{3}$	6	18	4.45 a.m.	39.7	0.812	0.675	40.2	0.815	0.760
9429	2	Holstein	$2\frac{11}{12}$	5	19.2	1 p.m.	Cloudy[1]	0.717	0.581	41.8	0.722	0.733
		Same sample, longer souring	......	....	....	....	41.0	0.717	0.648	....	....	....

TABLE 4.—*Continued.*

LABORATORY NUMBER	NUMBER OF COW	BREED	AGE OF COW	TIME SINCE CALVING	AMOUNT OF MILK	TIME OF MILKING	SPONTANEOUS SERUM			ACETIC ACID SERUM		
							Refraction at 20°C.	Ash	Lactic acid	Refraction at 20°C.	Ash × 1.02	Lactic acid
			years	*months*	*pounds*		*reading*	*gram per 100 cc.*	*gram per 100 cc.*	*reading*	*gram per 100 cc.*	*gram per 100 cc.*
9435	2	Holstein	2$\frac{11}{12}$	5	19.2	1 p.m.	40.7	0.709	0.497	41.2	0.709	0.779
		Same sample, longer souring	......	....	....	....	40.7	0.711	0.544	....	....	....
		Same sample, longer souring	......	....	....	....	40.7	0.702	0.596	....	....	....
9437	2	Holstein	2$\frac{11}{12}$	5	12.8	7.30 p.m.	40.7	0.671	0.554	40.7	0.722	0.767
		Same sample, longer souring	......	....	....	....	40.0	0.687	0.653	....	....	....
		Same sample, longer souring	......	....	....	....	40.2	0.722	0.713	....	....	....
9439	2	Holstein	2$\frac{11}{12}$	5	19.0	4.45 a.m.	41.7	0.699	0.544	42.0	0.717	0.751
9448	3	Holstein	5	3	20.7	p.m.	40.7	0.757	0.648	41.2	0.760	....
9453	3	Holstein	5	3	23.2	a.m.	41.2	0.757	0.770	41.6	0.752	....
9450	4	Holstein	9	9	11.8	p.m.	41.4	0.745	0.563	42.0	0.746	....
9454	4	Holstein	9	9	16.8	a.m.	40.7	0.737	0.648	41.7	0.736	....
9475	4	Holstein	9	9	13.0	p.m.	40.1	0.732	0.657	41.1	0.734	....
9449	5	Holstein	5	4	21.5	p.m.	41.3	0.724	0.535	41.7	0.714	....
9456	5	Holstein	5	4	27.5	a.m.	40.9	0.755	0.713	41.5	0.740	....
9457	6	Holstein	6	7	12.3	p.m.	42.5	0.739	0.619	41.2	0.773	....
9458	6	Holstein	6	7	19.6	a.m.	39.4	0.721	0.554	40.1	0.734	....
9474	6	Holstein	6	7	15.5	p.m.	Cloudy	0.709	0.488	39.8	0.716	....
9461	7	Holstein	6	8	8.6	p.m.	41.0	0.769	0.535	41.9	0.767	....
9462	7	Holstein	6	8		a.m.	41.5	0.712	0.666	40.5	0.726	....
9476	8	Holstein	4	2	20.0	p.m.	....	....	....	40.3	0.733	....
9443	9	Grade Holstein	8–9	Nearly dry	6.5	p.m.	40.7	0.813	0.742	40.7	0.830	....
9444	9	Grade Holstein	8–9	Nearly dry	6.5	a.m.	40.7	0.797	0.665	40.9	0.782	....
9452	10	Grade Holstein	......	3	13[2]	a.m.	41.7	0.729	0.657	41.8	0.741	....
9447	10	Grade Holstein	......	3	13[2]	p.m.	41.3	0.732	0.653	41.3	0.735	....

9446	11	Grade Holstein	8–9	10	4.3	a.m.	42.7	0.807	0.675	42.7	0.811	....
9451	12	Grade Holstein	6	10	16.8	a.m.	38.5	0.794	0.517	38.7	0.796	....
9455	12	Grade Holstein	6	10	17.7	p.m.	41.4	0.816	0.734	39.2	0.818	....
9459	13	Grade Holstein	......	8	5.4	p.m.	41.4	0.924[3]	0.657	Cloudy[4]	0.877	....
9460	13	Grade Holstein	......	8	8.0	a.m.	42.6	0.862	0.716	43.7	0.864	....
9464	14	Grade Holstein	6	¾	19.0	a.m.	40.3	0.822	0.733	42.7	0.816	....
9465	15	Grade Holstein	9	4	7.0	p.m.	39.1	0.776	0.619	38.0	0.775	....
9466	15	Grade Holstein	9	4	10.0	a.m.	39.2	0.797	0.657	38.8	0.783	....
9472	15	Grade Holstein	9	4	7.8	p.m.	37.9	0.766	0.517	37.8	0.769	....
9467	16	Grade Holstein	2	11	6	p.m.	39.9	0.744	0.601	40.5	0.741	....
9468	16	Grade Holstein	2	11	8.2	a.m.	40.0	0.767	0.657	40.0	0.745	....
9473	16	Grade Holstein	2	11	7.4	p.m.	40.5	0.745	0.657	40.9	0.753	....
9441	17	Durham	5	2	13.0	p.m.	40.7	0.774	0.742	41.2	0.775	....
9442	18	Durham	2½	1	....	p.m.	41.0	0.751	0.704	41.3	0.756	....
9470	19	Durham	6–7	¾	17.4	p.m.	41.9	0.756	0.751	42.5	0.736	....
9471	20	Durham	5	¾	17.4	p.m.	41.5	0.803	0.704	41.8	0.785	....
9440	21	Black Polled	2½	½	13.0	p.m.	39.7	0.763	0.666	40.1	0.786	....
9445	22	Black Polled	2	½	13.0	a.m.	41.7	0.762	0.733	42.2	0.814	....
9469	23	Grade Guernsey	3	1	13.0	p.m.	41.4	0.772	0.779	42.1	0.755	....
Minimum........							37.9	0.671	....	38.0	0.709	....
Maximum........							42.7	0.924	....	43.7	0.877	....
Mean........							40.6	0.765	....	41.0	0.769	....

[1] Could not get spontaneous serum clear enough to read.

[2] Approximately.

[3] Extra high results may have been due to slow filtering of very small amount of serum.

[4] Could not get filtrate clear enough to read.

TABLE 5.

Analysis of milks from individual cows. (*September–October, 1915.*)

(J. T. Keister, Washington, D. C.)

LABORATORY NUMBER	NUMBER OF COW	BREED	AGE OF COW	TIME SINCE CALVING	AMOUNT OF MILK	TIME OF MILKING	FAT (ROESE-GOTTLIEB METHOD)	FAT (BABCOCK METHOD)	SOLIDS NOT FAT (GRAVIMETRICALLY)	SOLIDS NOT FAT (CALCULATED)	SOUR SERUM		ASH
											Refraction at 20° C.	Ash	
			years	*months*	*pounds*		*per cent*	*per cent*	*per cent*	*per cent*	*reading*	*gram per 100 cc.*	*per cent*
9434	1[1]	Holstein	4½	6	15.2	1 p.m.	3.82	3.85	7.96	7.86	39.2	0.748	0.75
9436	1	Holstein	4½	6	12.5	7.30 p.m.	3.71	3.80	7.78	7.78	39.0	0.790	0.74
9438	1	Holstein	4½	6	18.0	4.45 a.m.	2.79	2.80	8.09	8.00	40.0	0.773	0.76
9435	2[1]	Holstein	$2\frac{11}{12}$	5	16.4	1 p.m.	4.22	4.30	8.18	8.12	40.9	0.678	0.657
9437	2	Holstein	$2\frac{11}{12}$	5	12.8	7.30 p.m.	3.88	3.95	8.12	8.00	40.3	0.668	0.66
9439	2	Holstein	$2\frac{11}{12}$	5	19.0	4.50 a.m.	2.55	2.70	8.52	8.35	40.8	0.704	0.669
9448	3	Holstein	5	3	20.7	p.m.	3.02	3.10	8.33	8.48	40.9	0.753	0.73
9453	3	Holstein	5	3	23.2	a.m.	3.01	3.10	8.44	8.33	41.3	0.764	0.72
9450	4	Holstein	9	9	11.8	p.m.	3.14	3.15	8.74	8.53	41.1	0.754	0.73
9454	4	Holstein	9	9	16.8	a.m.	2.48	2.50	8.76	8.50	40.8	0.756	0.71
9475	4	Holstein	9	9	13.0	3.40 p.m.	2.80	2.80	8.78	8.46	41.1	0.745	0.68
9449	5	Holstein	5	4	21.5	p.m.	3.07	3.15	8.35	8.41	40.4	0.769	0.73
9456	5	Holstein	5	4	27.5	a.m.	3.02	3.15	8.36	8.45	40.9	0.743	0.70
9457	6	Holstein	6	7	12.3	p.m.	3.77	3.90	8.64	8.40	41.3	0.787	0.706
9458	6	Holstein	6	7	19.6	a.m.	3.30	3.05	8.13	7.96	39.2	0.740	0.68
9474	6	Holstein	6	7	15.5	p.m.	....	4.20	....	8.05	38.0	0.731	0.664
9461	7	Holstein	6	8	8.6	p.m.	3.31	3.30	8.76	8.56	41.9	0.770	0.69
9462	7	Holstein	6	8	10.0	a.m.	3.21	3.30	8.53	8.31	40.4	0.731	0.74
9476	8	Holstein	4	2	20.0	p.m.	....	3.75	....	8.10	39.7	0.729	0.71
9443	9	Grade Holstein	8 or 9	8 or 9	8.7	p.m.	3.47	3.20	8.56	....	40.9	0.853	0.76
9444	9	Grade Holstein	8 or 9	8 or 9	8.7	a.m.	3.72	3.50	8.63	....	....	....	0.73
9447	10	Grade Holstein	3	3	17.4	p.m.	....	4.00	....	8.26	40.4	0.731	....
9452	10	Grade Holstein	....	3	17.4	a.m.	....	3.60	....	8.59	42.7	0.739	....
9446	11	Grade Holstein	8 or 9	8 or 10	4.3	a.m.	....	7.70	....	8.99	43.2	0.808	....

9451	12	Grade Holstein	6	10	16.8	p.m.	3.40	3.45	7.49	7.54	41.8	0.801	0.75
9455	12	Grade Holstein	6	10	17.7	a.m.	2.84	2.95	7.65	7.71	41.7	0.813	0.73
9459	13	Grade Holstein	....	8	5.4	p.m.	....	4.80	....	9.30	41.8	0.882	....
9460	13	Grade Holstein	....	8	8.0	a.m.	....	4.40	....	9.08	42.6	0.898	....
9464	14	Grade Holstein	6	3	19.0	a.m.	....	3.10	....	8.97	41.1	0.832	....
9465[2]	15	Grade Holstein	9	4	7.0	p.m.	(1.10)[2]	(1.10)	7.48	7.37	40.2	0.781	0.68
9466	15	Grade Holstein	9	4	10.0	a.m.	3.81	3.80	7.79	7.58	40.9	0.806	0.70
9472	15	Grade Holstein	9	4	7.8	p.m.	3.09	3.10	7.60	7.51	38.4	0.790	0.72
9467	16	Grade Holstein	2	11	6.0	p.m.	....	4.20	....	8.09	40.0	0.760	....
9468	16	Grade Holstein	2	11	8.2	a.m.	3.54	3.45	8.22	8.21	41.4	0.754	0.68
9473	16	Grade Holstein	2	11	7.4	p.m.	3.37	3.45	8.47	8.36	41.1	0.770	0.686
9441	17	Grade Durham	5	2	13.0	p.m.	....	3.75	....	8.46	40.8	0.782	....
9442	18	Grade Durham	2½	1	10.9	p.m.	....	3.50	....	8.55	41.0	0.745	....
9470	18	Grade Durham	6	3	17.4	p.m.	....	3.65	....	8.98	41.8	0.811	....
9471	20	Grade Durham	5	3	17.4	p.m.	....	3.80	....	8.55	41.8	0.761	....
9440	21	Black Polled	2½	2	13.0	p.m.	3.18	3.25	8.35	8.26	40.4	0.791	0.767
9445	21	Black Polled	2½	2	13.0	a.m.	3.10	3.20	8.86	8.68	41.9	0.814	0.79
9469	22	Grade Guernsey	3	1	13.0	p.m.	....	3.85	....	8.68	41.1	0.779	....
9478	23	Grade Jersey	20(about)	3	8.7	p.m.	2.83	2.80	....	8.33	40.4	0.806	....
9479	24	Grade Jersey	10	1	13.0	p.m.	....	4.80	....	7.81	40.1	0.816	0.75
9480	25	Grade Durham	5	1	10.9	p.m.	....	6.60[3]	....	8.57	40.6	0.869	....
9481	26	Grade Durham	10(about)	1	10.9	p.m.	....	7.90[3]	....	8.85	43.0	0.832	....
9482[4]	Herd	....	....	....	....	p.m.	....	4.60	....	8.42	40.7	0.790	....

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[1] Cows milked 3 times a day.

[2] Sample taken by milker, probably represents first portion of the milking.

[3] Only 8 hours since previous milking, which probably partly accounts for high fat figure.

[4] Herd test of 10 cows (Nos. 13, 17, 18, 19, 20, 21, 23, 24, 25, 26).

TABLE 6.

Composition of milk from Fagot Dairy, Metairie Ridge, Jefferson Parish.
(G. B. Taylor, J. R. Reeny and J. S. Slack, Louisiana.)

NAME	AGE	BREED	AMOUNT OF MILK	TIME SINCE CALVING	SP. GR. AT 15° C.	TOTAL SOLIDS[1]	FAT	SOUR SERUM	
								Ash	Refraction
	years		*pounds*	*months*		*per cent*	*per cent*	*gram per 100 cc.*	*reading*
Brown	8	Grade Jersey	3	9	1.0354	14.88	5.00	0.898	45.2
Nigger	8	Brown Grade Jersey	8	6	1.0344	15.11	5.40	0.876	43.6
Grandma	10	Holstein	8	½	1.0317	15.16	6.00	0.805	44.2
Hazel	3	Grade Jersey	4½	2	1.0328	14.95	5.60	0.788	43.4
Spot	5	Grade Jersey	7	5	1.0315	15.71	6.50	0.744	43.1
Rabbit	7	Grade Jersey	8	2	1.0325	14.63	5.40	0.787	43.9
Silver	3	Grade Jersey	4½	2	1.0302	14.89	6.10	0.707	42.3
Baby	6	Jersey	7	2	1.0260	......	8.20	0.880	43.1
Goldie	12	Jersey	5	6	1.0298	15.64	6.80	0.840	43.4
Red	8	Grade Jersey	5	6	1.0295	13.15	4.80	0.842	41.5
Brownie	5	Brown Grade Jersey	9	5	1.0298	15.40	6.60	0.844	44.4
Rose	10	Red Grade Jersey	8½	8	1.0293	13.82	5.40	0.874	43.6
Jessie	6	Jersey	12½	2	1.0315	14.14	5.20	0.744	42.0
Mollie	9	Holstein	6	10	1.0316	11.75	3.20	0.813	42.0
Marie	7	Jersey	5	10	1.0321	......	7.40	0.902	43.6
White	8	Grade Jersey	8	5	1.0351	14.57	4.80	0.866	43.6
Cherry	8	Grade Durham	9	4	1.0329	14.01	4.80	0.795	44.2
Crip	5	Grade Jersey	6	2	1.0322	14.55	5.40	0.820	41.7
Juliet	8	Grade Jersey	5½	4	1.0345	11.52	2.40	0.708	42.8
Composite[2]	......	....	29	......	1.0302	15.01	6.20	0.810	43.4
Herd[3]	......	....	129½	......	1.0315	13.89	5.00	0.805	42.8

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[1] U. S. Bur. Animal Industry Bull. 134, p. 23.

[2] Composite of 6 Grade Jerseys.

[3] Four Jerseys; 12 Grade Jerseys; 2 Holsteins; 1 Durham.

TABLE 7.

Composition of 8 samples of herd milk.
(Arranged in order of Total Solids.)

TOTAL SOLIDS	FAT	SOLIDS NOT FAT	PRO-TEIN	SUGAR	COPPER SERUM		SOUR SERUM	
					Ash	Refraction	Ash	Refraction
per cent	*per cent*	*per cent*	*per cent*	*per cent*	*gram per 100 cc.*	*reading*	*gram per 100 cc.*	*reading*
13.02	4.60	8.42	....	....	....	....	0.790	40.7
12.95	4.10	8.85	3.29	4.74	0.70	37.4	0.774	40.4
12.62	3.40	9.22	3.04	5.01	0.76	38.5	0.816	41.0
12.60	3.35	9.25	2.33	5.04	0.78	38.7	0.832	41.9
12.57	4.00	8.57	3.18	4.82	0.68	37.2	0.766	41.5
12.24	3.55	8.69	3.34	4.65	0.74	36.7	0.808	......
12.13	3.35	8.78	2.96	4.72	0.80	36.8	......	41.3
11.67	3.40	8.27	2.84	4.51	0.77	36.3	0.804	39.1
Maximum: 13.02	4.60	9.25	3.34	5.04	0.80	38.7	0.832	41.9
Minimum: 11.67	3.35	8.27	2.33	4.51	0.68	36.3	0.766	39.1
Average: 12.48	3.72	8.76	3.00	4.78	0.75	37.4	0.799	40.8
Average of 67 samples from herds reported in 1914.								
12.83	4.06	8.77	....	....	....	37.9	0.798	41.7

There is no relation between the refraction of the sour serum and the sour serum ash, since these figures depend upon different milk constituents. When both of these figures fall below the lowest limits established for pure milk (38.3 and 0.730) it is absolute proof of the presence of added water, and all possibility of the sample being abnormal milk from a sick cow is removed.

In the determination of the ash of sour serum there are less than 2 grams of organic matter to be burned, and the influence of combustion upon the 190 mg. of ash is very slight.

The refractive index of the sour serum occurs half way between the copper and acetic indices. Where a milk has partially soured the copper serum reading will be higher, and the acetic serum lower than would be given by the same milk before souring. In such cases the sour serum is the only reliable reading to be made.

Six years' experience with the refractive index and 4 with the sour serum ash have shown these methods to be invaluable. In all doubtful cases the sour serum ash has served as a court of last resort.

RECOMMENDATIONS.

It is recommended—

(1) That the following be adopted as auxiliary provisional methods.

DETECTION OF ADDED WATER.

Sour serum[1].

Allow the milk to sour spontaneously, filter and determine the index of refraction of the clear serum by means of the Zeiss immersion refractometer. A reading below 38.3 indicates added water.

Ash of sour serum[2].

Allow the milk to sour spontaneously and filter. Transfer 25 cc. of this serum to a flat-bottomed platinum dish and evaporate to dryness over the water bath. Heat the contents of the dish over a small flame (to avoid sputtering) until charred. Place the dish in an electric muffle, with pyrometer connected, and ash at a heat not greater than 500°C., or 900°F. Cool and weigh. Express result in grams per 100 cc. An ash below 0.730 indicates added water. (A white ash is invariably obtained after one leaching.)

Ash of acetic serum.

Transfer 25 cc. of the serum to a flat-bottomed platinum dish and proceed as directed under "Ash of Sour Serum". An ash figure below 0.715 gram per 100 cc. indicates added water.

(2) That in conjunction with the copper, acetic or sour serum refraction method, the ash of the sour serum or of the acetic serum be determined in all cases where the indices of refraction fall below the minimum limit. The acetic serum ash multiplied by the factor 1.021 equals the sour serum ash (dilution of the acetic serum being 2%).

No report was made by the referee on the general subject of food adulteration.

REPORT ON COLORS.

By W. E. Mathewson (Food and Drug Inspection Laboratory, U. S. Appraiser's Stores, New York, N. Y.), *Associate Referee.*

Two samples of commercial caramel and sealed samples of high grade commercial amaranth, orange I, naphthol yellow S, and light green S F yellowish were sent out for collaborative work. The collaborating analysts were asked to examine the caramels in 0.2% solution by those methods for the detection of this substance that they consider best. In the coal tar dyes they were asked to determine the amount of color by the titanium trichlorid reduction method of Knecht and Hibbert which has the merit of using a comparatively simple reagent, stable if protected from the air, and which has given good results in the New York Food and Drug Inspection Laboratory of the U. S. Bureau of Chemistry when used under the proper precautions and with a thorough consciousness of the sensitiveness of the reagent to air. With the samples was sent a statement of the precautions to be followed in applying the method to each of the permitted dyes. These are embodied in the suggested provisional methods which come before the Association this year and need not be given here.

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[1] Matthes and Müller. Z. öffent. Chem., 1903, **9**: 173.

[2] Burr and Berberich. Chem. Ztg., 1908, **32**: 617–8.

The collaborators reported upon the caramel samples as follows:

RESULTS OF COLLABORATIVE WORK.

C. F. Jablon: The samples of caramel under observation did not behave alike to the Amthor test. In solution of 0.2%, one gave a slight precipitate, while the other gave a pronounced precipitate. In stronger solutions (1%) a slight turbidity was observed in the first, while the other showed a pronounced precipitate.

The Woodman-Newhall test does not appear to give any more reliable results. In weaker solutions (0.2%) it appears to be less satisfactory, while a 1% solution gave positive reactions. To sum up the series of experiments, it is safe to state that although strong caramel solutions gave positive tests, ordinary concentrations, such as may be found in food analysis, do not give very satisfactory results.

Ray W. Clough: A few qualitative tests on the caramel samples gave the following results:

Crampton and Simons fuller's earth method[1], all color extracted from a 0.2% solution; Amthor test, negative; Woodman-Newhall method, good results (especially heavy precipitate with the phenylhydrazin reagent).

C. R. Smith: The Woodman-Newhall method was applied to the samples of caramel. It has been found that they, as well as other samples of commercial caramel and caramel prepared in the laboratory, precipitate out well in the Woodman-Newhall method. It is doubtful if the same holds good in fruit juices and other food products. In testing black currant juice of high acidity no precipitate was produced, using the usual proportion of reagents after neutralizing with potash.

By adding a large excess of zinc chlorid (8 cc.) and potash sufficient to produce a good precipitate a large amount of brownish black coloring matter was carried down, which, however, did not precipitate by the paraldehyde test. The Woodman-Newhall test applied with special reference to each substance may be useful if carefully studied and developed.

The results obtained by the collaborators in the determination of the amount of color in the samples of commercial anilin colors are given in Table 1.

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[1] U. S. Bur. Chem. Bull. 107, Rev., p. 199.

TABLE 1.

Determinations of the amount of pure color in samples of anilin colors by titration with titanium trichlorid.

ANALYST	NAPHTHOL YELLOW S	ORANGE I	AMARANTH	LIGHT GREEN S F YELLOWISH
	per cent	*per cent*	*per cent*	*per cent*
C. L. Black, Food and Drug Inspection Laboratory, U. S. Appraiser's Stores, Philadelphia, Pa.	93.28	90.82	88.39	66.44
A. L. Burns, Food and Drug Inspection Laboratory, U. S. Appraiser's Stores, New York, N. Y.	92.9	91.7	88.6	66.8
C. F. Jablon, Food and Drug Inspection Laboratory, U. S. Appraiser's Stores, New York, N. Y.	92.7	91.0	88.7	67.1
Ray W. Clough, Arcade Annex Building, Seattle, Wash.	93.4	93.8	88.8	66.1
C. R. Smith, Food and Drug Inspection Laboratory, U. S. Appraiser's Stores, New York, N. Y.	93.1	93.2	89.1	67.9

THE SEPARATION OF PONCEAU 3R FROM OTHER DYES, ESPECIALLY NAPHTHOL YELLOW S BY THE USE OF A SOLUBLE BARIUM SALT.

(C. F. Jablon, New York.)

As the quantitative separation and estimation of ponceau 3R and naphthol yellow S is, under ordinary conditions, rather slow, a method was sought whereby the more or less tedious amyl alcohol extraction would be eliminated.

Inasmuch as ponceau 3R forms a very difficultly soluble barium compound[1] a series of experiments were performed using barium and strontium salts as precipitants in solutions containing the dyes in various concentrations. The best results were obtained as follows:

Dissolve the dye mixture in a definite amount of water. Transfer an aliquot containing about 0.1–0.3 gram of naphthol yellow S to a 250 cc. volumetric flask and dilute with water to about 225 cc. Add 2.0 cc. of 10% barium acetate and 1 cc. of glacial acetic acid, shake thoroughly and make up to the mark with water. Then pass through a dry filter and titrate an aliquot with standard titanium trichlorid solution in sodium tartrate solution, using light green S F yellowish as indicator. Titrating an equivalent part of the original solution gives the total color, and subtracting the yellow, the difference gives the value for ponceau 3R.

Table 2 gives some results obtained.

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[1] Heumann. Die Anilinfarben und ihre Fabrikation. 1903, **4** (I): 856.

TABLE 2.

Determinations by the barium method of naphthol yellow S in presence of ponceau 3R.
(C. F. Jablon, New York.)

SAMPLE	WEIGHT PONCEAU TAKEN	WEIGHT YELLOW TAKEN	WEIGHT YELLOW FOUND	YELLOW RECOVERED
	gram	*gram*	*gram*	*per cent*
1	0.100	0.2491	0.2496	100.2
2	0.200	0.2076	0.2050	98.7
3	0.300	0.2030	0.2028	99.9
4	0.200	0.1833	0.1819	99.2
5	0.100	0.1538	0.1555	101.2
6	0.300	0.1005	0.0998	99.0
7	0.200	0.0240	0.0258	107.6
8	0.300	0.0000	0.0013	.....

The filtrate from the precipitated ponceau 3R was somewhat redder in shade, so it was deemed advisable to determine colorimetrically with a Koenig-Martens instrument the amount of ponceau remaining in solution. Following are the amounts found, using various concentrations of ponceau 3R:

Weight of ponceau taken	Ponceau in solution
gram	*per cent*
0.05	2.25
0.125	1.4
0.250	0.5
0.500	0.2
1.000	0.1

The incomplete precipitation is probably due to the fact that the ponceau 3R used was not a chemically uniform product. It was the ordinary grade of dye used for food products which contains more or less of the lower homologues derived from xylidin, etc., and also small amounts of monosulphonated color such as sodium trimethyl-benzene-azo-2-naphthol-6-sulphonate.

Mixtures of ponceau 3R with the other permitted dyes in amounts varying from 0.1–0.3 gram of each dye when treated by the procedure just described showed that erythrosine is precipitated almost completely, and the indigo somewhat less so with ponceau. Amaranth and orange I are carried down with the ponceau precipitate only slightly, if at all. Light green S F yellowish appears to remain entirely in the filtrate.

Because of the high relative oxidizing power of naphthol yellow S as shown towards titanium trichlorid, the error caused by the presence of a small and varying amount of ponceau in the filtrate does not greatly affect the determination of the yellow with mixtures containing not too small amounts of the latter. With the other dyes this is not the case, but the results stated may be applied to the analysis of mixtures containing ponceau and naphthol yellow with the others. Here the total dye in the filtrate is determined with titanium, the amaranth, green, etc., estimated directly by some suitable method, and correction made to obtain the value for the yellow. With lesser accuracy, the procedure may be used for the direct separation of ponceau from orange, amaranth and green. The quantitative determination of indigo carmine in admixture with amaranth by reduction with titanium trichlorid, reoxidation with air, and estimation of the blue colorimetrically seems to give good results.

Ponceau and yellow being separated with some difficulty by other methods and often used in admixture with each other, the procedure just given will be found useful in examining mixtures.

THE USE OF THE SPECTROPHOTOMETER FOR THE EXAMINATION OF FOOD COLORING MATTER.

(W. E. Mathewson, New York.)

No other methods have the wide range of applicability of those depending on exact measurements under suitable conditions of the opacity of solutions to light of various wave lengths. The principles and methods of spectrophotometry have been developed by numerous investigators and are discussed in an extensive literature[1]. These methods are in limited use, probably because the necessary instruments are so expensive that few laboratories are equipped with them. Hence no collaborative work could be done this year. In this part of the report some experimental work of the referee is reported which was designed to learn to what extent spectrophotometric methods might be used in the analysis of food colors. As each of the various optical systems that have been devised and each of the light sources available has its advantages and disadvantages, such apparatus as seemed most suitable was selected.

APPARATUS AND PROCEDURE.

The spectrophotometer used was a Koenig-Martens instrument with Rutherford prism, made by Schmidt and Haensch. Some experience with a spectrophotometer of the Vierort principle has convinced the writer that those forms of apparatus in which an eye-piece is employed (forming a system focused on the collimator slit) are much more difficult to use in work where it is very frequently necessary to take readings in parts of the spectrum in which the absorption varies greatly for small variations in the wave length. As a light source a 1500–3000 c. p. projection lamp with automatic arc and condensing lenses was employed. A Nernst lamp although steadier can scarcely be used for measurements in the blue and violet. Monochromatic light sources suitable for all regions of the spectrum would be much more preferable, but would add greatly to the cost of the equipment. An absorption cell of the Vierort type was used, 11 mm. in width and containing the Schultz glass cube exactly 1 cm. in thickness. This form of cell is rather objectionable for measurements in non-homogeneous light, as all the rays passing into the instrument must go through 1 mm. of the solution; but the laboratory is not provided with the better apparatus devised by Martens and Grünbaum[1]. The cell was placed before the instrument, the glass cube first in front of one slit, then the other. The ocular slit or screen in the telescope is rather troublesome to get at and adjust in the Koenig-Martens instrument, and chiefly for this reason was set at a width corresponding to 100 (smallest) divisions on the scale carrying the telescope (at the mercury line 546.1), and kept at this width for all the measurements. To correspond, the telescope was moved 100 scale divisions between the successive regions. The jaws of the divided slit before the collimating lens were set as close as possible, still giving a field of sufficient brightness, and the observations made with the glass cube before each half of the slit after each resetting of the latter. The necessary opening of the collimator slit corresponded to a maximum overlapping in the field of view of rays differing by about 2.5 $\mu\mu$ in the indigo and violet to 0.1 $\mu\mu$ in the greenish yellow. In all cases the width of the slit was noted in taking measurements, though for Table 3, containing averaged figures, the statements just made are sufficient. For each reading the rotating nicol prism was set 2 or 3 times and the mean value recorded. The results were calculated to "extinction coefficients" e, by the formula

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[1] For a résumé compare especially Kayser. Handbuch der Spectroscopie. 1905, **3**; and G. and H. Kruss. Kolorimetrie und Quantitative Spektrolana yse. 2d ed., 1909.

$$e = \log \tan a_1 + \log \cot a_2$$

a_1 and a_2 representing respectively the angular readings with the Schulz body before the right and left parts of the slit. For the loss of light by reflection at the surfaces of the Schulz body the correction for water was found to be 0.02, this amount to be added to the observed "extinction coefficient". In Table 3, this correction has not been made, however.

EXPERIMENTAL RESULTS.

Extinction coefficients of the permitted dyes.—In Table 3 are given the observed "specific extinction coefficients" obtained with solutions of the 7 permitted dyes, corresponding to the values for 0.001% solutions. Solutions of each color were made containing 0.001%, 0.004%, 0.008%, etc., of pure dye as determined by analysis, the strongest solution being from 0.064–0.512%. The extinction coefficients for each solution were determined in those regions where the values ranged from 0.030–1.50. From these figures the *e* values for 0.001% solutions through the whole spectrum were calculated, it being assumed that the observed figures of value equal to about 0.60, if not more accurate, were at least more suitable for subsequent application.

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[1] Ann. Physik., 1903, 4th ser., **12**: 991–2.

Light green S F yellowish being quite sensitive to alkali, dilute solutions in pure water if kept in glass soon became paler and in 24 hours may show a conversion of 50% or more of the dye into the colorless carbinol form. Orange I and erythrosine are also sensitive indicators and may contain as impurities small amounts of sodium carbonate, free color acid, etc. This source of difficulty was practically eliminated by adding to each solution a little of a mixture of acetic acid and secondary sodium phosphate. A stock solution was made containing in each liter 71.4 grams of crystallized secondary sodium phosphate and 9.6 cc. of glacial acetic acid. Five cc. of this solution were used in making up each 100 cc. of the dye solutions, giving these latter a disodium phosphate normality of 0.01, and an acetic acid normality of 0.008 plus. This amphoteric mixture is faintly acid to litmus, faintly alkaline to alizarin and does not perceptibly change the absorption of the solutions of the colors other than light green S F yellowish, when added to their absolutely neutral solutions in not too large quantities.

The extinction coefficients as determined are not exactly proportional to concentration, being affected by a number of factors. The molecular condition of both electrolytes and colloids varies with changes in concentration, and the use of non-homogeneous light is open to various objections. However, the figures serve as a guide, indicating especially where are located those regions of maximum absorption best adapted for measurements, and the values to be expected. The accurate estimation of a dye must be made by determining the approximate concentration of its solution by the use of Table 3, changing the concentration, if necessary, so that the values for *e* will be from 0.6–1.00 in the region of maximum absorption, making a standard solution of the same calculated concentration from a pure color and determining the absorption constants for each under exactly the same conditions. The values obtained for *e* will not differ greatly and it may be assumed under these conditions that the differences are proportional to concentration.

Analysis of mixtures.—The analysis of mixtures must be carried out in a similar way. After quantitative examination of the constituents by chemical, spectroscopic or other means, measurements are made in those regions where the constituent dyes show the maximum absorption. The concentration is calculated, using the figures in Table 3, and from this a similar standard solution is prepared with colors of known purity. This is

TABLE 3.

Specific extinction coefficients of the 7 permitted food dyes.

(0.01 gram per liter is taken as unit concentration; i.e., the numbers given represent the coefficients of 0.001% solutions.)

WAVE LENGTH OF LIGHT IN REGION MEASURED	SCALE READINGS	NAPHTHOL YELLOW S	ORANGE I	PONCEAU 3R (FROM COMMERCIAL PSUEDOCUMIDIN)	ERYTHROSINE	AMARANTH	INDIGO-CARMINE	LIGHT GREEN S F YELLOWISH
418.8–422.6	22	0.46	0.30	0.13	0.027	0.093	0.029	0.18
422.6–426.5	23	0.46	0.32	0.13	0.028	0.095	0.029	0.18
426.5–430.7	24	0.46	0.38	0.13	0.028	0.105	0.030	0.18
430.7–435.1	25	0.45	0.45	0.13	0.029	0.11	0.031	0.17
435.1–440.0	26	0.45	0.52	0.14	0.030	0.115	0.032	0.15
440.0–444.5	27	0.44	0.58	0.155	0.034	0.125	0.033	0.13
444.5–449.6	28	0.40	0.67	0.17	0.040	0.13	0.034	0.105
449.6–455.0	29	0.34	0.73	0.19	0.052	0.14	0.034	0.077
455.0–460.7	30	0.29	0.80	0.23	0.068	0.165	0.034	0.056
460.7–466.7	31	0.23	0.87	0.265	0.085	0.19	0.034	0.034
466.7–473.1	32	0.16	0.91	0.30	0.12	0.215	0.034	0.023
473.1–480.0	33	0.105	0.93	0.34	0.17	0.25	0.034	0.015
480.0–487.1	34	0.060	0.90	0.39	0.24	0.29	0.035	0.011
487.1–494.8	35	0.031	0.84	0.43	0.32	0.33	0.038	0.011
494.8–503.0	36	0.014	0.71	0.48	0.37	0.37	0.043	0.013
503.0–511.8	37	0.0051	0.59	0.48	0.48	0.40	0.053	0.017
511.8–521.2	38	0.0024	0.43	0.46	0.76	0.41	0.066	0.024
521.2–531.4	39	0.00092	0.27	0.425	0.97	0.41	0.090	0.036
531.4–542.3	40	0.00031	0.17	0.37	0.59	0.38	0.125	0.055
542.3–554.2	41	0.00005	0.092	0.24	0.16	0.32	0.17	0.086
554.2–567.1	42	Less	0.043	0.11	0.032	0.24	0.23	0.14
567.1–581.1	43	....	0.019	0.045	0.010	0.14	0.30	0.23
581.1–596.4	44	....	0.0072	0.017	0.0023	0.054	0.37	0.36
596.4–613.3	45	....	0.0024	0.0056	0.0004	0.016	0.46	0.53
613.3–631.9	46	....	0.0008	0.0018	0.0001	0.0046	0.41	0.81
631.9–652.5	47	....	0.0003	0.0004	....	0.0013	0.023	0.77
652.5–675.6	48	....	0.0001	0.0001	....	0.0003	0.075	0.30
675.6–701.4	49	....	....	....	....	0.0002	0.022	0.069
701.4–730.6	50	....	....	....	....	....	0.007	0.010
622.4–642.0	46.5	....	....	....	....	....	....	0.88

examined under the same conditions. If, as should be the case, the *e* values do not differ more than 10%, the figures given in Table 3 are shown to be sufficiently correct under the applied conditions to be used for calculating the corrections to be made, these being deduced from the figures in the table and the differences between the *e* values for the known and the unknown solutions. With mixtures, especially, the use of nonhomogeneous light is a cause of differences and useful results can hardly be expected without comparative solutions. The figures given for light in the violet, of wave length less than 430$\mu\mu$, are quite inaccurate, and for analytical purposes it would be scarcely advisable to make measurements beyond 440$\mu\mu$.

Most binary mixtures may be analyzed with fair ease and accuracy. With more complicated ones, it has been found that usually one or more of the constituents may be determined with advantage in this way, but that the analysis is best made partly by other methods.

In Table 4 are given the results of 5 consecutive test analyses of mixtures of 2 components.

TABLE 4.

Spectrophotometric analysis of mixtures of 2 dyes.
(W. E. Mathewson, New York.)

DYES	CONCENTRATION GRAM PER 100 CC.	PER CENT OF TRUE VALUE FOUND BY FIRST CALCULATION (USING FIGURES OF TABLE 3)	PER CENT OF TRUE VALUE FOUND AFTER MAKING COMPARISON SOLUTIONS AND DETERMINING CORRECTION
Naphthol yellow S	0.005625	101.0	101.7
Light green S F yellowish	0.002375	95.6	100.4
Indigo carmine	0.001800	92.6	99.1
Amaranth	0.002200	101.0	100.7
Orange I	0.002000	106.0	102.0
Ponceau 3R	0.001440	94.4	96.7
Orange I	0.000800	99.0	101.0
Naphthol yellow S	0.007200	100.1	99.0
Amaranth	0.002000	97.8	98.4
Erythrosine	0.001200	102.3	100.0

Maximum error 3.3%; average error 1.3%.

The calculations with binary mixtures may be made more easily graphically than algebraically.

Determination of coal tar dyes other than those permitted.—The optical method permits a good quantitative estimation with extremely small amounts of color, and has been found to be well suited for the determination of such non-permitted dyes as orange II, acid yellow G, tartrazine and rose bengal in food products.

Determination of natural coloring matters used for food products.—Very few of these coloring matters can be estimated even approximately by chemical methods. Even where the coloring principle has never been isolated in purity, the method still allows an expression of the amount of color in terms of the extinction coefficients and permits standards to be set for such food coloring products as caramel, archil, etc.

A number of samples of saffron examined in the New York Food and Drug Inspection Laboratory of the U. S. Bureau of Chemistry have shown a wide variation in tinctorial power. One gram of the drug was macerated with occasional shaking for 24 hours with 200 cc. of alcohol, exactly 50% by volume. Two cc. of this solution were measured off, diluted to 100 cc. with 50% alcohol, and the values for *e* determined. The value of the extinction coefficient at 461–467$\mu\mu$ varied from 0.72–1.73.

A few analyses have indicated that curcumine in turmeric root is readily determined by extraction of the powdered drug with 95% alcohol in a Soxhlet apparatus, dilution of the extract so that the ratio to the weight of the turmeric taken is about 10,000: 2 and determination of the extinction coefficients of the solution. These may be compared with the values obtained with pure crystallized curcumine under the same conditions.

REPORT ON SACCHARINE PRODUCTS.

DETECTION OF ARTIFICIAL INVERT SUGAR IN HONEY.

By F. L. Shannon (Dairy and Food Department, Lansing, Mich.), *Associate Referee.*

The work this year was a continuation of work previously inaugurated. Last year the referee selected 9 of the tests the literature showed to be most commonly used. These 9 tests were submitted to the collaborators along with samples and each test studied. From this work the referee for 1914 recommended "That four of the tests which proved the most satisfactory in the hands of all the collaborators be further studied". Accordingly instructions for these 4 tests were sent with the samples to the collaborators.

PREPARATION OF SAMPLES.

Seven samples were prepared as follows:

Sample A.—Pure honey plus 3% commercial invert sugar[1].
Sample B.—Pure honey plus 5% commercial invert sugar.
Sample C.—Pure honey plus 10% commercial invert sugar.
Sample D.—Pure honey.
Sample E.—Pure honey plus 3% Neumoline[2].
Sample F.—Pure honey plus 5% Neumoline.
Sample G.—Pure honey plus 10% Neumoline.

INSTRUCTIONS TO COLLABORATORS.

It is requested that the following tests be applied to the samples submitted:

FIEHE'S ORIGINAL TEST[3].

Reagent.—Dissolve 1 gram of resorcinol in 100 cc. of HCl (1.19). Redistilled ether.

Manipulation.—Rub 1 gram of honey in a mortar with ether. Filter off the ether and evaporate at room temperature. Moisten the residue with a drop of the reagent.

Results.—In the presence of artificial invert sugar an orange-red color is developed, changing to cherry-red and then to brown-red. Pure honeys sometimes give a pink coloration. Note color after standing 10 minutes and again after standing 24 hours.

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[1] Obtained on the market and sold as such.

[2] A commercial product put on the market as a non-crystallizable sirup and also a substitute for honey.

[3] Analyst, 1908, **33**: 397.

HARTMANN'S MODIFICATION OF FIEHE'S TEST[1].

Reagent.—Same as original Fiehe Test.

Manipulation.—Add 2 drops of the reagent directly to 1 gram of the honey in a porcelain dish.

Results.—If artificial invert sugar is present, a cherry-red color appears as in the original test. Natural honeys give the reaction after standing about 45 minutes.

BRYAN'S MODIFICATION OF FIEHE'S TEST[2].

Reagent.—Same as original Fiehe Test.

Manipulation.—Place 10 cc. of a 50% honey solution in a test tube and run in 5 cc. of ether on top. Shake contents gently and allow to stand for some time until the ether layer is perfectly clear; transfer 2 cc. of this clear ether solution to a small test tube and add a large sized drop of the resorcin solution. Shake and note immediately the color.

Results.—In the presence of artificial invert sugar the drop of added acid in the bottom assumes immediately an orange-red color, turning to a dark red.

FEDER'S ANILIN CHLORID TEST[3].

Reagent (freshly prepared).—To 100 cc. of C. P. anilin add 30 cc. of 25% hydrochloric acid.

Manipulation.—Mix directly 5 grams of the honey in a porcelain dish with 2.5 cc. of the anilin reagent.

Results.—Bright red color indicates the presence of artificial invert sugar. The intensity of the color is proportional to the amount present.

Give your opinion, based on the foregoing tests, as to which samples are adulterated, if any, and which are pure, if any. Report results on each test on each sample as positive, negative or doubtful.

TABLE 1.
Results of collaborators on Sample A.
(Pure honey plus 3% commercial invert sugar.)

ANALYST	FIEHE'S ORIGINAL TEST		HARTMANN'S MODIFICATION OF FIEHE'S TEST		BRYAN'S MODIFICATION OF FIEHE'S TEST	FEDER'S ANILIN CHLORID TEST
	10 minutes	24 hours	10 minutes	24 hours		
S. F. Sherwood, Bureau of Chemistry, Washington, D. C.	Very strong trace	Very strong trace	Trace	Very dark cherry-red	Trace	Very slight trace
J. O. Clark, Department of Agriculture, Atlanta, Ga.	Positive	Positive	Positive	Positive	Positive	Positive
W. L. Scovill, Dairy and Food Department, Lansing, Mich.	Positive	Positive	Positive	Positive	Positive	Positive
N. A. Childs, Dairy and Food Department, Lansing, Mich.	Positive	Positive	Positive	Positive	Positive	Positive
F. L. Shannon, Dairy and Food Department, Lansing, Mich.	Positive	Positive	Positive	Positive	Positive	Positive
C. G. Sutton, Dairy and Food Department, St. Paul, Minn.	Positive	Positive	Positive	Positive	Positive	Positive

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[1] Z. Nahr. Genussm, 1911, **21**: 374.

[2] U. S. Bur. Chem. Bull. 154, p. 15.

[3] Analyst, 1911, **36**: 586.

TABLE 2.

Results of collaborators on Sample B.
(Pure honey plus 5% commercial invert sugar.)

ANALYST	FIEHE'S ORIGINAL TEST		HARTMANN'S MODIFICATION OF FIEHE'S TEST		BRYAN'S MODIFICATION OF FIEHE'S TEST	FEDER'S ANILIN CHLORID TEST
	10 minutes	24 hours	10 minutes	24 hours		
S. F. Sherwood..........	Very strong trace	Very strong trace	Strong trace	Verydark cherry-red	Strong trace	Trace
J. O. Clarke.............	Positive	Positive	Positive	Positive	Positive	Positive
W. L. Scovill............	Positive	Positive	Positive	Positive	Positive	Positive
N. A. Childs............	Positive	Positive	Positive	Positive	Positive	Positive
F. L. Shannon...........	Positive	Positive	Positive	Positive	Positive	Positive
C. G. Sutton............	Positive	Positive	Positive	Positive	Positive	Positive

TABLE 3.

Results of collaborators on Sample C.
(Pure honey plus 10% commercial invert sugar.)

ANALYST	FIEHE'S ORIGINAL TEST		HARTMANN'S MODIFICATION OF FIEHE'S TEST		BRYAN'S MODIFICATION OF FIEHE'S TEST	FEDER'S ANILIN CHLORID TEST
	10 minutes	24 hours	10 minutes	24 hours		
S. F. Sherwood..........	Very strong trace	Very strong trace	Very strong trace	Very dark red	Very strong trace	Very strong trace
J. O. Clarke.............	Positive	Positive	Positive	Positive	Positive	Positive
W. L. Scovill............	Positive	Positive	Positive	Positive	Positive	Positive
N. A. Childs............	Positive	Positive	Positive	Positive	Positive	Positive
F. L. Shannon...........	Positive	Positive	Positive	Positive	Positive	Positive
C. G. Sutton............	Positive	Positive	Positive	Positive	Positive	Positive

TABLE 4.

Results of collaborators on Sample D.
(Pure honey.)

ANALYST	FIEHE'S ORIGINAL TEST		HARTMANN'S MODIFICATION OF FIEHE'S TEST		BRYAN'S MODIFICATION OF FIEHE'S TEST	FEDER'S ANILIN CHLORID TEST
	10 minutes	24 hours	10 minutes	24 hours		
S. F. Sherwood....	Negative	Doubtful	Negative	Very dark red	Negative	Negative
J. O. Clarke.......	Negative	Negative	Slight pink Doubtful	Positive	Negative	Negative
W. L. Scovill......	Negative	Positive	Negative	Positive	Negative	Negative
N. A. Childs......	Negative	Negative	Doubtful	Positive	Negative	Negative
F. L. Shannon.....	Negative	Negative	Negative	Positive	Negative	Negative
C. G. Sutton......	Negative	Negative	Positive Faint pink	Positive	Negative	Negative

TABLE 5.

Results of collaborators on Sample E.
(Pure honey plus 3% Neumoline.)

ANALYST	FIEHE'S ORIGINAL TEST		HARTMANN'S MODIFICATION OF FIEHE'S TEST		BRYAN'S MODIFICATION OF FIEHE'S TEST	FEDER'S ANILIN CHLORID TEST
	10 minutes	24 hours	10 minutes	24 hours		
S. F. Sherwood	Negative	Doubtful	Very slight trace	Very dark color	Very slight trace. Several minutes to develop color	Negative
J. O. Clarke	Positive	Positive	Positive	Positive	Positive	Positive
W. L. Scovill	Doubtful	Positive	Doubtful	Positive	Positive	Positive
N. A. Childs	Doubtful	Negative	Doubtful	Positive	Doubtful	Doubtful
F. L. Shannon	Negative	Positive	Negative	Positive	Positive	Doubtful
C. G. Sutton	Positive Very faint pink	Positive	Positive	Positive	Positive	Positive Light pink

TABLE 6.

Results of collaborators on Sample F.
(Pure honey plus 5% Neumoline.)

ANALYST	FIEHE'S ORIGINAL TEST		HARTMANN'S MODIFICATION OF FIEHE'S TEST		BRYAN'S MODIFICATION OF FIEHE'S TEST	FEDER'S ANILIN CHLORID TEST
	10 minutes	24 hours	10 minutes	24 hours		
S. F. Sherwood	Doubtful	Trace	Very slight trace	Very dark red	Very slight trace	Doubtful
J. O. Clarke	Positive	Positive	Positive	Positive	Positive	Positive
W. L. Scovill	Positive	Positive	Positive	Positive	Positive	Positive
N. A. Childs	Positive	Positive	Positive	Positive	Positive	Positive
F. L. Shannon	Positive	Positive	Positive	Positive	Positive	Positive
C. G. Sutton	Positive Faint pink	Positive	Positive	Positive	Positive	Positive Pink

TABLE 7.

Results of collaborators on Sample G.
(Pure honey plus 10% Neumoline.)

ANALYST	FIEHE'S ORIGINAL TEST		HARTMANN'S MODIFICATION OF FIEHE'S TEST		BRYAN'S MODIFICATION OF FIEHE'S TEST	FEDER'S ANILIN CHLORID TEST
	10 minutes	24 hours	10 minutes	24 hours		
S. F. Sherwood	Trace	Strong trace	Trace	Very dark red	Trace	Trace
J. O. Clarke	Positive	Positive	Positive	Positive	Positive	Positive
W. L. Scovill	Positive	Positive	Positive	Positive	Positive	Positive
N. A. Childs	Positive	Positive	Positive	Positive	Positive	Positive
F. L. Shannon	Positive	Positive	Positive	Positive	Positive	Positive
C. G. Sutton	Positive	Positive	Positive	Positive	Positive	Positive

TABLE 8.

Conclusion as to purity of samples.

ANALYST	SAMPLE A	SAMPLE B	SAMPLE C	SAMPLE D	SAMPLE E	SAMPLE F	SAMPLE G
F. Sherwood.....	Adulterated	Adulterated	Adulterated	Pure	Doubtful	Doubtful	Adulterated
O. Clarke.......	Adulterated	Adulterated	Adulterated	Pure	Doubtful	Adulterated	Adulterated
. L. Scovill.......	Adulterated	Adulterated	Adulterated	Pure	Doubtful	Adulterated	Adulterated
. A. Childs.......	Adulterated	Adulterated	Adulterated	Pure	Doubtful	Adulterated	Adulterated
L. Shannon.....	Adulterated	Adulterated	Adulterated	Pure	Pure	Adulterated	Adulterated
G. Sutton.......	Adulterated	Adulterated	Adulterated	Pure	Adulterated	Adulterated	Adulterated

COMMENTS OF COLLABORATORS.

S. F. Sherwood: The color developed in Feder's test is very uncertain. Hartmann's modification is liable to give the color reaction in pure honeys and hence be misleading. Bryan's modification is preferable to Fiehe's original test, as a more thorough extraction of the honey is obtained and less time required. The color should be developed at once. Bryan's modification is preferable to any of the others. It has been used by the writer frequently and with satisfactory results.

J. O. Clarke: All reactions on Sample No. 5 were somewhat faint, but sufficient to be classed as "positive".

W. L. Scovill: In Bryan's modification of Fiehe's test it is preferable to add 2 drops of the reagent instead of 1. The 24 hour test with the original Fiehe reaction and Hartmann's modification is not characteristic, as a pure honey will give a positive reaction.

The original Fiehe test and Hartmann's modification give slight positive reactions with pure honey.

F. L. Shannon: There is no advantage in either Fiehe's original or in Hartmann's modification in allowing them to stand 24 hours and then drawing conclusions. The color is well developed in 2 minutes and the best conclusion can be drawn at the end of that time.

DISCUSSION.

The results of the collaborators on these 4 tests indicate that it is not possible in all cases to detect artificial invert sugar in honey when the adulteration is less than 5%. It is also evident that the possibility of detecting artificial invert sugar in honey depends upon the nature of the invert sugar present. None of the collaborators hesitated to pronounce Samples A, B and C adulterated although Sample A contained but 3% adulterant, while in the case of Sample E, which contained but 3% adulterant, all were doubtful as to its purity, although not quite positive that it was adulterated. Sample A contained 3% of one kind of invert sugar and Sample E 3% of another.

All of these reactions depend upon furfural or its derivatives and the intensity of the color developed depends upon the amount of furfural present. This may be large or small, depending upon the manner in which the artificial invert sugar has been made. No accurate conclusions can be drawn, therefore, from any of the tests as to the amount of the adulterant present.

Nothing is gained by allowing any of the tests to stand for any length of time before drawing conclusions. The color in all instances is developed at once when the adulteration is perceptible.

RECOMMENDATIONS.

It is recommended—

(1) That Bryan's modification of Fiehe's test, as given on page 150, be adopted as provisional.

(2) That Fiehe's test as given on page be 149, adopted as provisional, except that the sentence "Note color after standing 10 minutes and again after standing 24 hours", be stricken out.

(3) That Feder's anilin chlorid test, as given on page 150, be adopted as provisional, except that the sentence "The intensity of the color is proportional to the amount present", be stricken out.

The Association adjourned at 11.55 to reassemble at 1 p.m.

MONDAY—AFTERNOON SESSION.

REPORT ON FRUIT PRODUCTS.

By P. B. Dunbar, *Associate Referee*, and H. A. Lepper (Bureau of Chemistry, Washington, D. C.).

The work on fruit products during the past year has been directed principally to a further study of methods for the determination of malic and citric acids. The associate referee for 1914, Mr. H. C. Gore, recommended a continued study of his proposed methods for the determination of these acids, and this recommendation has been followed in the case of the malic-acid method. The present referee has been unable to secure any details of the proposed method for citric acid and consequently no work on this method has been undertaken. A study has been made, however, of the modification of Stahre's method[1] for the determination of citric acid, recently published by Kunz.[2] The "Methods for the analyses of fruits and fruit products,"[3] have also been reviewed, and a critical study of the modified Schmidt-Hiepe method for tartaric, citric, and malic acids[4] has been made. It has not seemed advisable to send out collaborative samples during the present year, as the details of the methods under study have not been completely worked out.

CITRIC ACID.

A somewhat detailed abstract of the method proposed by Kunz for the determination of citric acid has already been published.[5] The quantitative method described is applicable only to the determination of citric acid in wines presumably dry. Methods for its qualitative detection in milk, marmalade, and fruit sirup are also described.

The quantitative method as applied to wine consists in treating the concentrated sample with sulphuric acid and bromin water, followed by a solution of potassium bromid. The solution is then oxidized with potassium permanganate, when insoluble pentabromacetone is precipitated quantitatively. While the method appears promising for the determination of citric acid in dry wines and other materials containing small amounts of permanganate reducing substances, it cannot be applied directly to fruit juices on account of the presence of large amounts of sugar. It, therefore, becomes necessary in order to apply the method to adopt some procedure for the precipitation of citric acid and its removal from most of the other permanganate reducing bodies. Precipitation as barium citrate from alcoholic solution has been advocated by many investigators, and seems to offer the most practical method of procedure.

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[1] Nordisk Tidsskrift (1895), **2**: 141; Z. anal. Chem. (1897), **36**: 195.

[2] Arch. Chem. Mikros (1914), **7**: 285–299.

[3] U. S. Bur. Chem. Bul. 107 (rev.), pp. 77–82.

[4] Ibid., pp. 80–81.

[5] Chem. Abstracts (1915), **9**: 687.

The method as finally adopted for study differs little, except for the fact that the citric acid is previously precipitated as barium citrate, from that recommended by Kunz for the determination of citric acid in wine. The procedure used for precipitating the acid is essentially that recommended for malic acid by the previous referee, Mr. Gore, but a solution of barium hydroxid is used instead of the solid substance, and the pectins are not removed before precipitation of barium citrate.

The method is as follows:

Determine the total acidity of the sample. It is convenient to use 1% azolitmin (made by dissolving 1 gram azolitmin in 80 cc. hot water, diluting to 100 cc. with 85% alcohol, and filtering) as an indicator in conjunction with a porcelain spot plate.

Weigh out 25 grams of the sample and transfer to a 600 cc. beaker with 95% alcohol; add an amount of approximately 0.25 N/1 barium hydroxid solution just sufficient to neutralize the acidity of the sample and 5 drops of 50% barium acetate solution, to insure an excess of barium. Dilute to a total volume of 375 cc. with 95% alcohol, stir well and allow to settle. After the precipitates of pectins and barium salts have settled well, decant the supernatant liquid into another beaker. Filter the decanted liquid through a folded filter to remove any small portions of precipitate which it may contain and wash the beaker with 95% alcohol. Filter the remainder of the pectin and barium salt precipitate on the same paper and wash the beaker and paper once with 95% alcohol. Transfer the precipitate quantitatively from the paper to the original beaker with hot water, boil until no more alcohol can be detected by odor, add enough sulphuric acid (1:5) to precipitate all of the barium originally added and to allow 2 cc. of the acid in excess, evaporate by careful boiling to between 60 and 70 cc., cool and add 5 cc. of freshly prepared saturated bromin water; the solution should show an excess of bromin. Transfer quantitatively to a 100 cc. graduated flask and dilute to the mark at room temperature; allow the precipitate of barium sulphate and that caused by the addition of bromin to settle and filter. The precipitate may be separated by centrifuging, and the supernatant liquid decanted if necessary. Now pipette an aliquot of the filtrate, containing not more than 400 mg. of citric acid, calculated from the total acidity of the sample, into a 300 cc. Erlenmeyer flask. The amount of citric acid in the aliquot should, if possible, exceed 50 mg. Add 10 cc. sulphuric acid (1 :1) and 5 cc. of potassium bromid solution (15 grams KBr in 40 cc. water). After shaking, warm the flask in a water bath to 48–50°C. and allow it to remain in the bath for five minutes. After removing from the bath, add 25 cc. of 5% $KMnO_4$ from a burette in rapid drops with frequent interruptions and constant vigorous shaking, care being taken that the temperature during oxidation does not exceed 55°. Set the flask aside until the hydrated peroxid of manganese begins to settle. (The supernatant liquid should be dark brown in color, showing an excess of $KMnO_4$. If an excess is not indicated, more $KMnO_4$ must be added.) Then shake and again set aside to settle, and repeat this operation until the precipitate takes on a yellow color and most of it has dissolved. Finally, while the solution is still warm, remove the last undissolved portion of the hydrated peroxid of manganese precipitate and also the excess of bromin by adding drop by drop a clear concentrated solution of ferrous sulphate acidified with H_2SO_4. Allow the solution to cool, with occasional shaking. If the operations have been properly carried out, a heavy white precipitate of pentabromacetone is obtained. This becomes crystalline on occasional shaking and in this condition is entirely insoluble in water. After the precipitate has become crystalline, preferably after standing overnight, collect it by means of gentle suction on a porcelain Gooch crucible provided with a thin pad of asbestos, previously dried over H_2SO_4 in a vacuum desiccator, and wash with distilled water slightly acidified with H_2SO_4 and, finally, twice with distilled water (if the precipitate has a tendency to pass through the filter, 1% H_2SO_4 should be used for washing). Dry the precipitate to constant weight in a vacuum desiccator over H_2SO_4 protected from strong light. The weight of pentabromacetone multiplied by the factor 0.464 equals citric acid plus 1 molecule of water of crystallization. It sometimes happens that the pentabromacetone is obtained in the form of oily droplets. These also become crystalline on standing, or on cooling, but are usually discolored by negligible traces of manganese or iron. There appears to be no difference in the results obtained whether the precipitate is originally in the form of crystals or oily droplets, which later become crystalline.

The accuracy of this method has been tested by determinations on water solutions of citric acid containing varying amounts of sucrose, and on diluted apple sirup and raspberry sirup containing added known amounts of citric acid, and in some cases added malic and tartaric acids. The results obtained are given in Table 1.

According to Partheil and Hübner,[1] 100 grams of alcohol of specific gravity 0.8092 dissolves 0.00578 gram of barium citrate $(C_6H_5O_7)_2 Ba_3 + 7H_2O$) at 25°C.

In determining small percentages of citric acid, the solubility of barium citrate in the volume of alcohol used will give rise to a relatively large error in the percentage recovered. Considerable time has been given to attempts to obviate this difficulty by determining the citric acid without previous precipitation, but these have been unsuccessful. It is hoped that the difficulty may be met by the use of a solubility correction. It is hardly necessary to point out, however, that a relatively small variation in the actual weight of citric acid found will give a marked variation in the percentage recovered and that comparisons should be made between the actual weights, in grams per 100 cc., of citric acid present and found. From the results on sirups containing added malic and tartaric acids, it appears that these acids do not interfere with the determination. This has been further shown by applying the method to 50 cc. portions of 1% solutions of malic, tartaric, and succinic acids. In no case was a precipitate obtained.

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[1] Arch. der Pharm., **241**: 412–435; Chem. Centr. (1903), **74**: (2), 1026.

This method seems of sufficient promise to warrant further study, and it is recommended that the referee for the following year be instructed to take up this work and, if possible, to send out collaborative samples.

TABLE 1.

Determination of citric acid by Kunz modification of Stahre's method.

CITRIC ACID IN AQUEOUS SOLUTION.

NO PRELIMINARY PRECIPITATION AS BARIUM CITRATE.

CITRIC ACID PRESENT		CITRIC ACID RECOVERED		
In 50 cc. sample	Per 100 cc.			
gram	*gram*	*gram*	*gram per 100 cc.*	*per cent*
0.0184	0.04	0.013	0.03	75.0
0.0908	0.18	0.086	0.17	95.2
0.0923	0.18	0.089	0.18	100.0
0.1845	0.37	0.181	0.36	97.3
0.1853	0.37	0.180	0.36	97.3
0.2270	0.45	0.223	0.45	98.3
0.2779	0.56	0.275	0.55	98.2
0.3706	0.74	0.370	0.74	100.0
0.4632	0.93	0.461	0.92	98.9

PRECIPITATION AS BARIUM CITRATE INCLUDED.

CITRIC ACID PRESENT		APPROXIMATE AMOUNT SUCROSE ADDED PER 100 CC.	CITRIC ACID RECOVERED		
In 25 cc. sample	Per 100 cc.				
gram	*grams*	*grams*	*grams*	*grams per 100 cc.*	*per cent*
0.4610	1.84	0	0.4503	1.80	97.68
0.4610	1.84	0.5	0.4491	1.80	97.42
0.4610	1.84	1.0	0.4493	1.80	97.47
0.4610	1.84	2.0	0.4487	1.79	97.33
0.4610	1.84	5.0	0.4465	1.79	96.85
0.4610	1.84	10.0	0.4448	1.78	96.50
0.4610	1.84	20.0	0.4455	1.78	96.64

ADDED CITRIC ACID IN DILUTED APPLE SIRUP.

CITRIC ACID PRESENT		APPROXIMATE MALIC ACID PRESENT	CITRIC ACID RECOVERED		
In 25 cc. sample	Per 100 cc.				
gram	*grams*	*per cent*	*gram*	*grams per 100 cc.*	*per cent*
0	0	0.51	0	0	0
0.0369	0.15	0.03	0.029	0.12	80.0
0.0484	0.19	0.53	0.038	0.15	78.9
0.0554	0.22	0.03	0.049	0.20	90.9
0.0727	0.29	0.53	0.059	0.24	82.8
0.0923	0.37	0.03	0.081	0.32	86.5
[1]0.0969	0.39	0.53	0.082	0.33	84.6
[2]0.0969	0.39	0.53	0.082	0.33	84.6
0.1211	0.48	0.53	0.109	0.44	91.7
0.1845	0.74	0.03	0.178	0.71	96.0
0.2707	1.08	0.51	0.243	0.97	89.8
0.4060	1.62	0.51	0.406	1.62	100.0

[1] 2.5 grams sucrose added to 25 cc. sample.
[2] 5 grams sucrose added to 25 cc. sample.

TABLE 1.—*Continued.*

ADDED CITRIC ACID IN RASPBERRY SIRUP.

CITRIC ACID PRESENT		APPROXIMATE MALIC ACID ADDED	APPROXIMATE TARTARIC ACID ADDED	CITRIC ACID RECOVERED[3]		
In 25 cc. sample	Per 100 cc.					
gram	*gram*	*per cent*	*per cent*	*gram*	*gram per 100 cc.*	*per cent*
[4]0.1373	0.55	0	0	0.155	0.62	112.7
[4]0.1373	0.55	0	0	0.154	0.62	112.7
[5]0.0992	0.40	0	0	0.097	0.39	97.5
[5]0.0992	0.40	0.39	0	0.094	0.38	95.0
[5]0.0992	0.40	0	0.40	0.097	0.39	97.5
[5]0.0992	0.40	0.19	0.20	0.093	0.37	92.5

[3] The last four determinations are corrected for the amount of citric acid found in the original sirup by precipitation.

[4] Calculated from titration of original sirup.

[5] Grams of citric acid added in excess of that originally present.

MALIC ACID.

The previous referee, Mr. Gore, has given considerable attention to a study of methods for the determination of malic acid during the past three years. In his report for the year 1912[1] he gives results obtained by eight collaborators, using the polariscopic method proposed by Dunbar and Bacon.[2] This method depends on the change in the rotation of malic-acid solutions when treated with uranyl acetate. The sample of cider analyzed contained 0.50% of total acidity calculated as malic. The results reported varied from 0.468 to 0.531%, and averaged 0.49%. During the following years, Mr. Gore paid considerable attention to the effects, on the rotations of uranium-malic acid solutions, of various factors. He further investigated the possibility of developing a method based on the change in rotation of malic-acid solutions when treated with ammonium heptomolybdate. As a result of this work he suggested, in his report for 1914, two polariscopic methods for the determination of malic acid, both of which call for a preliminary precipitation of the acid as barium malate.

The uranyl-acetate method suggested differs from the Dunbar-Bacon method, first sent out by Mr. Gore for collaborative work, in that precipitation of malic acid is suggested, a solution of uranyl acetate is used in place of the solid salt, and the amounts of malic acid are determined by referring the polariscopic reading to a table, rather than by the use of a constant factor. The method of Dunbar and Bacon is not applicable in the presence of tartaric acid nor in the case of highly colored liquids which cannot be readily polarized. A method whereby the malic acid may be precipitated and separated from interfering substances is therefore highly desirable. The use of a solution of uranyl acetate in place of the solid salt is also advantageous in that it obviates the indefinite error of dilution, due to the solution of unknown amounts of uranyl acetate. In the case of products containing small amounts of malic acid, however, the dilution of the solution with an equal volume of uranyl-acetate solution produces a corresponding decrease in the polarization and thereby increases the relative percentage error due to inaccuracies in reading the polariscope.

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[1] U. S. Bur. Chem. Bul. 162, p. 63.

[2] U. S. Bur. Chem. Circ. 76.

The 1914 report on fruit products contained results by only two collaborators using the proposed methods for malic acid suggested by Mr. Gore. These, unfortunately, do not agree very satisfactorily, and it seemed advisable, therefore, to carry out further experimental work with solutions containing known amounts of malic acid. Numerous difficulties were encountered in precipitating the acid; and, although considerable time was given to the work, the results so far obtained are not such as to justify a final recommendation regarding the method. It was found to be difficult, if not impossible, to dissolve the solid barium hydroxid which is added to precipitate the malic acid, and a strong standardized barium-hydroxid solution was therefore substituted. The error due to the solubility of barium malate does not seem to be serious. It was found, however, that in the case of solutions containing malic and tartaric acids, a sufficient amount of barium tartrate was dissolved to reduce the negative polarization somewhat in the uranyl-acetate method and increase it in the molybdate method. The irregularities of results due apparently to this cause are shown in Table 2. Comparison of the actual weights of malic acid present with those found, however, show that the error in grams per 100 cc. is not large. In the case of determinations of malic acid in fruit juices, much of the color of the original sample is carried down with the barium precipitate and again dissolved when this precipitate is taken up in water.

TABLE 2.

Determination of malic acid by the uranyl-acetate and molybdate methods in aqueous solutions containing tartaric acid.

MALIC ACID PRESENT		TARTARIC ACID PRESENT IN 25 CC. SAMPLE	MALIC ACID RECOVERED					
In 25 cc. sample	Per 100 cc.		By uranyl-acetate method			By molybdate method		
gram	*gram*	*gram*	*gram*	*gram per 100 cc.*	*per cent*	*gram*	*gram per 100 cc.*	*per cent*
0.0938	0.38	0.0997	0.092	0.37	97.4	0.102	0.41	107.9
0.0938	0.38	0.1496	0.088	0.35	92.1	0.100	0.40	105.2
0.1407	0.56	0.0997	0.130	0.52	92.9	0.157	0.63	112.5
0.1876	0.75	0.0499	0.165	0.66	98.0	0.223	0.89	118.6

It thus appears that the method suggested for the precipitation of barium malate does not entirely obviate the difficulties in the original method when interfering color is present, but is of promise in determining malic acid in the presence of tartaric when the color of the solution is not excessive. In the absence of tartaric acid and color the precipitation method introduces a number of time-consuming operations without materially affecting the results.

The work which was undertaken on citric acid prevented an extended study of the malic-acid method. From the results of the collaborative work reported by Mr. Gore in 1912 and those obtained by the present referee and reported in U. S. Bureau of Chemistry Circular 76, it would appear that the malic-acid method as originally described in that circular can undoubtedly be applied with accuracy to solutions which can be polarized readily and which contain no optically active acid other than malic. Where the amount of malic acid present is sufficient, the use of a solution of uranyl acetate is apparently to be preferred to the solid salt. When it is possible to make readings without difficulty, rapid determination may be made with reasonable accuracy without previous neutralization or clarification by polarizing before and after treatment with uranyl acetate. In the presence of tartaric acid, preliminary precipitation as the barium salt is advantageous.

REVIEW OF METHODS FOR THE ANALYSES OF FRUITS AND FRUIT PRODUCTS.

A number of recommendations for changes in the methods for the analyses of fruits and fruit products have been forwarded to the chairman of the committee on the revision of methods. These recommendations will doubtless be included in the report of the committee.

TABLE 3.

Experiments with the Schmidt-Hiepe method for tartaric, citric, and malic acids.[1]

NO.	TARTARIC ACID			CITRIC ACID			MALIC ACID		
	Added	Recovered		Added	Recovered		Added	Recovered	
	gram	*gram*	*per cent*	*gram*	*gram*	*per cent*	*gram*	*gram*	*per cent*
1	0.4896	0.4834	98.73	0.4601	0.3955	85.96	....	0.0304	....
2	0.4896	0.4884	99.76	....	0.0019	....	0.4856	0.4000	82.37
3	....	0.0195	....	0.4462	0.2957	66.27	0.4856	0.5832	120.1
4	0.3917	0.3856	98.44	0.3570	0.1760	49.30	0.3885	0.4536	116.8
5	0.1469	0.0706	48.06	0.1339	0.0803	59.97	....	0.0586	....
6	0.1469	0.0702	47.78	....	0.0025	....	0.1457	0.1286	88.29
7	....	0.0075	....	0.1339	0.0141	10.53	0.1457	0.1631	112.0
8	0.0979	0.0957	97.75	0.0920	0.0118	12.83	0.1003	0.0141	13.06
9	0.0490	0.0342	69.80	0.0460	0.0109	23.69	....	lost	....
10	0.0490	0	0	....	0.0102	....	0.0502	0	0
11	....	0.0045	....	0.0460	0.0048	10.43	0.0502	lost	....
12	0.0490	0.0305	62.24	0.0460	0	0	0.0502	lost	....

[1] U. S. Bur. Chem. Bul. 107 (rev.), p. 80.

In 3, 7, and 11, where a recovery is recorded for tartaric acid when none was present, the titer was probably caused by calcium acetate retained, due to the limitation to the washing.

The amount of tartaric acid recovered in 3, 7, and 11 is the amount actually titrated, the 0.0286 gram dissolved in the 100 cc. filtrate not being added, as no tartaric was present.

The amount of malic recovered was always corrected for the tartaric (0.0286) present when it was known to be there, due to original addition.

Table 3 contains the results of a study of the modified Schmidt-Hiepe method for the determination of tartaric, citric, and malic acids. From these results it appears that the method is unreliable, and should be dropped as one of the official methods.

RECOMMENDATIONS.

It is recommended—

(1) That the Kunz modification of Stahre's method for the determination of citric acid be further studied.

(2) That the uranyl-acetate and ammonium heptomolybdate methods for the determination of malic acid be further studied.

(3) That the method for "Tartaric, citric, and malic acids (Schmidt-Hiepe method modified) official"[1] be dropped.

REPORT ON WINE.

By B. G. Hartmann (Bureau of Chemistry Food and Drug Inspection Laboratory, Chicago, Ill.), *Associate Referee.*[2]

The provisional method for the determination of total tartaric acid content in wines,[3] is inaccurate and unreliable. In a paper read before the association in 1912, Hartmann and Eoff called attention to this fact, presenting proof in support of their criticisms and offering for trial a modification of this method. This paper gave the initiative for a series of coöperative investigations extending over a period of three years, conducted for the purpose of determining the relative merits of the two methods as to their accuracy and adaptability to varying conditions. In the course of these investigations the methods were tried on wines, grape juices, and synthetic solutions containing varying amounts of tartaric and phosphoric acids.

The results of this work proved conclusively that the provisional method is far from satisfactory, whereas the proposed method was found to be accurate and reliable.

During the present year five samples of grape juice, with and without the addition of tartaric acid and phosphoric acid, were sent to collaborators with the following instructions:

INSTRUCTIONS TO COLLABORATORS.

The samples were filtered and no precipitation is expected. However, as a precautionary measure, shake the samples thoroughly just before measuring the various portions.

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[1] U. S. Bur. Chem. Bul. 107 (rev.), pp. 80–81.

[2] Presented by M. J. Ingle.

[3] U. S. Bur. Chem. Bul. 107 (rev.), p. 86.

ACIDITY OF THE JUICE.

Measure 10 cc. of the sample into a 250 cc. beaker, add 20 cc. distilled water and titrate with tenth-normal sodium hydroxid until two drops of the solution when mixed on a porcelain tile with several drops of a neutral azolitmin solution give no red tint. Prepare the azolitmin solution by dissolving 0.5 gram pure azolitmin in a liter of distilled water, neutralizing with either acid or alkali, as the case may be.

DETERMINATION OF TOTAL TARTARIC ACID.

Provisional method.—Determine as described in U. S. Bureau of Chemistry Bulletin 107 (revised), page 86, using 50 cc. of sample, and dilute with 50 cc. distilled water. Use 20 cc. of alcohol instead of 15 cc. Collect the cream of tartar crystals on a double filter paper in a Büchner funnel (see U. S. Bureau of Chemistry Bulletin 162, p. 73) and titrate with N/10 alkali, using phenolphthalein as indicator. Multiply by 2 to obtain grams per 100 cc. Do not stir longer than 1 minute and do not place in an ice box for the 15 hours prescribed, but allow to remain at room temperature.

Proposed method (Hartmann and Eoff[1]*).*—Transfer 50 cc. of the sample to a 250 cc. beaker and neutralize with sodium hydroxid. The amount of normal alkali required is calculated by multiplying the cc. N/10 sodium hydroxid required to titrate the 10 cc. of the sample (under 1) by 0.5. To the neutralized solution, add enough water to make 100 cc. and add tartaric acid. The tartaric acid used should be of the purest obtainable and should be powdered and dried for about two hours at the temperature of boiling water. Determine the purity of the tartaric acid by titrating 0.30 gram with N/10 alkali, using phenolphthalein as indicator. Keep the tartaric acid in a desiccator. The amount of tartaric acid to be added is calculated by multiplying the number of cc. N/1 NaOH required to neutralize the 50 cc. sample by 0.075. Weigh the amount accurately.

After the tartaric acid has gone into solution, add 15 grams powdered potassium chlorid and stir until the salt has dissolved. Now add 2 cc. glacial acetic acid and 20 cc. of 95% alcohol. Stir until precipitation has started and place in an ice box for at least 15 hours. Collect the cream of tartar crystals on a double filter paper in a Büchner funnel and titrate with N/10 alkali, using phenolphthalein as indicator. Calculate the total tartaric acid and subtract the tartaric added, making proper correction for purity of the latter. This will give the tartaric acid in 50 cc. Multiply by 2 to obtain grams per 100 cc.

The basis of the five samples was a pure Concord grape juice. In order to prevent precipitation of potassium acid tartrate, the filtered juice was diluted with 25% of distilled water. The samples were made up as follows:

No. 1.—The diluted juice.

No. 2.—The diluted juice with the addition of 0.569 gram of tartaric acid per 100 cc.

No. 3.—The diluted juice with the addition of 0.06 gram of phosphoric acid per 100 cc.

No. 4.—The diluted juice with the addition of 0.30 gram of phosphoric acid per 100 cc.

No. 5.—The diluted juice with the addition of 0.086 gram sodium carbonate to neutralize the free tartaric acid.

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[1] U. S. Bur. Chem. Bul. 162, p. 72.

All samples were sterilized.

The following results were obtained by the collaborators and the associate referee.

Total tartaric acid (grams per 100 cc.).

ANALYST	SAMPLE 1		2		3		4		5	
	No. 1	No. 2	No. 1	No. 2	No. 1	No. 2	No. 1	No. 2	No. 1	No. 2
J. R. Eoff, Jr.	0.528	0.614	0.912	1.210	0.504	0.614	0.414	0.628	0.555	0.624
	0.534	0.608	0.924	1.208	0.507	0.620	0.408	0.620	0.558	0.620
M. J. Ingle	0.558	0.627	0.969	1.205	0.542	0.636	0.433	0.638	0.590	0.637
F. D. Merrill	0.578	0.628	1.024	1.213	0.579	0.586	0.382	0.586	0.562	0.602
	....	0.628	1.024	......	......	......	......	......	0.568	0.606
E. H. Berry	0.556	0.626	0.960	1.200	0.544	0.613	0.444	0.627	0.582	0.615
	0.564	0.629	0.964	1.205	0.550	0.625	0.450	0.633	0.585	0.615
T. G. Gleason Edw. F. Higgins	0.538	0.618	0.952	1.200	0.526	0.636	0.435	0.644	0.584	0.632
B. G. Hartmann	0.562	0.618	0.960	1.190	0.544	0.613	0.438	0.618	0.586	0.618
	0.568	0.623	0.972	1.195	0.550	0.620	0.442	0.625	0.588	0.620
Average	0.554	0.622	0.966	1.203	0.538	0.618	0.427	0.624	0.576	0.619

No. 1, provisional method; No. 2, proposed method.

No comments on the two methods were offered by the collaborators. M. J. Ingle submitted the results which he obtained on the five samples by the Rochelle-salt method, as follows:

> The following are the results obtained by using Rochelle salt on the five samples submitted. I did not add any alkali to these determinations, merely a quantity of Rochelle salt calculated by multiplying the number of cc. of N/1 alkali required for complete neutralization by the factor 0.141. The portions of the juice used were measured on July 19 and had to be thoroughly shaken to mix in the precipitated tartar. I then added 50 cc. of water, 2 cc. acetic, 15 grams KCl, and 20 cc. of 95% alcohol, and placed in the ice box over night. I ran these determinations in duplicate and obtained good checks. I found out that one cannot be certain of the state of hydration of these salt crystals, particularly if grinding is resorted to. It will therefore be necessary to run a blank in duplicate on 1-gram samples of the salt as used. The tartaric acid recovered will represent the factor for obtaining the percentage of acid added from the weight of Rochelle salt used.
>
> The procedure in the case of the blank consisted in merely diluting to 100 cc. and adding the acetic and other constituents, omitting, as above, the potassium acetate.

Total tartaric acid (grams per 100 cc.).

	Rochelle-salt method	Proposed method
No. 1	0.624	0.627
No. 2	1.170	1.205
No. 3	0.624	0.636
No. 4	0.620	0.638
No. 5	0.640	0.637

DISCUSSION OF RESULTS.

The general average of the results obtained by the collaborators for the two methods are:

Total tartaric acid (grams per 100 cc.).

	Provisional method	Proposed method
No. 1	0.554	0.622
No. 2	0.966	1.203
No. 3	0.538	0.618
No. 4	0.427	0.624
No. 5	0.576	0.619

With the exception of sample No. 2, the total tartaric-acid content of the five samples submitted is identical. Taking as a basis the general average (0.621) of the results obtained on these four samples by the proposed method, the recoveries by the provisional method are:

	Per cent
No. 1 (containing free tartaric acid)	89
No. 3 (containing 0.06 gram phosphoric acid)	87
No. 4 (containing 0.30 gram phosphoric acid)	70
No. 5 (containing no free tartaric acid)	93

Sample No. 2 had an addition of 0.569 gram of tartaric acid per 100 cc. Of this amount the proposed method recovered 102% and the provisional method 61%.

From these results it is evident that the provisional method is unreliable and inaccurate and that the proposed method gives very satisfactory results. Regarding the provisional method the results show—

(1) That it fails to determine all the free tartaric acid.

(2) That the addition of potassium acetate has a decomposing effect upon potassium-acid tartrate.

(3) That the presence of a free mineral acid such as phosphoric acid partially prevents the formation of potassium-acid tartrate.

The first two points, the presence or absence of free tartaric acid, are the two conditions occurring in natural wines and grape juices, whereas the presence of added phosphoric acid is very often met with in artificial grape products, such as grape-juice sirups and other soda-fountain beverages. Accordingly, the provisional method is not applicable to any of the conditions which occur in wines or grape juices, unless it is assumed that a relative amount of free tartaric acid retards the decomposing action of the potassium acetate upon potassium-acid tartrate:

$$\mathrm{KAc} + \mathrm{H_2T} = \mathrm{KHT} + \mathrm{HAC}.$$

In conclusion it should be said that the deductions made from the results obtained in this investigation are similar to those made in the three previous reports on this subject.

In making a recommendation for the adoption of the proposed method, it seems desirable to call attention to the fact that many results have been published on authentic samples of wines and grape juices of known purity examined by the provisional method. Results obtained by the proposed method would not, therefore, be comparable with previously published figures. It does not seem, however, that a method found to be entirely unsatisfactory should be retained by the association.

RECOMMENDATION.

It is recommended—

That the provisional method be dropped by the association and the proposed method be adopted for the determination of total tartaric acid in wines, grape juice, and soda-fountain sirups.

The following is the method for wines:

Neutralize 100 cc. wine with normal sodium hydroxid. The amount of alkali necessary for neutralization is calculated from the acidity of the wine. If the volume of the solution is increased more than 10% by the addition of the alkali, evaporate the solution to approximately 100 cc. Add tartaric acid to the neutralized solution. For each cubic centimeter normal alkali used add 0.075 gram tartaric acid.[1] After the tartaric acid has dissolved, add 2 cc. glacial acetic acid and 15 grams potassium chlorid. After the potassium chlorid has dissolved, add 15 cc. of 95% alcohol, stir vigorously until the cream of tartar starts to precipitate, and let stand for at least 15 hours in an ice box. Decant the liquid from the separated acid-potassium tartrate on a Gooch prepared with a very thin film of asbestos or on filter paper in a Büchner funnel. Wash the precipitate and filter three times with a small amount of mixture of 15 grams of potassium chlorid, 20 cc. of 95% alcohol, and 100 cc. of water, using not more than 20 cc. of the wash solution in all. Transfer the asbestos or paper and precipitate to the beaker in which the precipitation took place, wash out the Gooch or Büchner funnel with hot water, add about 50 cc. of hot water, heat to boiling, and titrate the hot solution with tenth normal sodium hydroxid, using phenolphthalein as indicator. Increase the number of cubic centimeters of N/10 alkali required by 1.5 cc. on account of solubility of the precipitate. One cubic centimeter of N/10 alkali is equivalent to 0.015 gram tartaric acid. To ascertain the tartaric acid originally present in the wine, subtract the amount of tartaric acid added. This will give the grams of total tartaric acid per 100 cc. of wine.

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[1] The tartaric acid used should be of the purest obtainable and should be powdered and dried for about two hours at the temperature of boiling water.

In the case of grape juice or sirups, use 50 cc. of the sample, neutralize with normal sodium hydroxid, and add distilled water to 100 cc. volume. Proceed as under wine, adding 20 cc. of alcohol instead of 15 cc. After subtracting the tartaric acid added, multiply by 2 to obtain grams total tartaric acid in 100 cc.

REPORT ON BEER.

By H. S. Paine (Bureau of Chemistry, Washington, D. C.),
Associate Referee.[1]

No plan for further work was submitted in the last report of the associate referee on this subject and, owing to a combination of circumstances, a plan for work which was deemed to be sufficiently profitable was devised too late for submittal to the collaborators.

Briefly, the matter may be stated as follows: Comparison of the analyses of various malt beverages, correlated with a knowledge of the conditions of the mash, leads to the conclusion that the methods employed for the determination of maltose (or "reducing sugars") and dextrin do not yield accurate results in all cases—the degree of accuracy being variable—that is, fairly acceptable in some cases and quite unsatisfactory in others. This variation is probably dependent upon the proportion of maltose and dextrin, and this in turn depends, of course, upon the mash conditions.

In the method which is based upon polarization there is a grave question of the accuracy of the assumed rotatory power of dextrin. There are various dextrins, of course, and it is a question whether the assumed value is always representative.

In the reduction method, which is more reliable, the factors which must be considered are somewhat as follows:

(1) The effect of dextrin and other constituents upon the reducing action of maltose in the reduction before hydrolysis, i.e., the question of applicability of the maltose tables to these conditions.

(2) The possibility of some reducing action by the lower dextrins.

(3) The possible effect of other sugars which might be present.

(4) The accuracy of the factors employed.

Various other considerations have a possible effect.

It is impossible in the absence of some preliminary experimental work to say just what the effect of these various factors may be and which will be of the most importance. The point to be emphasized, however, is that the variations in the composition of the mash and conditions of mashing are the controlling factors which cause a variable degree of accuracy in the above determinations. The other methods employed in beer analysis are, on the whole, satisfactory.

It is believed that the carbohydrate determinations are most in need of attention at the present time, and it is recommended that the methods for the determination of maltose and dextrin in beer be made the subject of study for the coming year.

No report was made by the associate referee on distilled liquors.

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[1] Presented by M. J. Ingle.

A STUDY OF THE FUSEL OIL AND ESTERS IN DISTILLED LIQUORS.

By Louis Katz (Bureau of Chemistry Food and Drug Inspection Laboratory, New York).[1]

The examination of brandies and other distilled liquors for fusel oil is ordinarily limited to an estimation of the quantity of fusel oil present. No attempt is made to gain an insight into the composition of the fusel oil. Considering the importance of the fusel oil constituent of distilled liquors as a basis of judgment to be pronounced by the chemist as to their genuineness, and further considering the comparative ease with which the recognized minimum quantity of fusel oil could be added to neutral spirits, it seemed quite obvious that a mere quantitative determination of fusel oil is a somewhat weak link in the chain of evidence the chemist gathers in the examination of liquors. At best it enables the chemist to pronounce a liquor sophisticated only in that limited number of cases where not enough of a so-called bona-fier or too much neutral spirits have been added to keep the quantity of fusel oil above the required minimum. A qualitative examination of the fusel oil of distilled liquors, supplementary to its quantitative determination, seemed highly desirable and was accordingly undertaken at the suggestion of Mr. A. F. Seeker. The property of the volatile acids of the acetic acid series, when fractionally distilled from an aqueous solution, to pass over at a rate which is definite and characteristic for each acid,[2] was made the basis of the study. This property of the individual acids causes any definite mixture of them to distil over at a rate which is definite and characteristic for that particular acid mixture. In the regular course of analysis of distilled liquors, the so-called fusel oil, or the sum total of the higher alcohols, is determined quantitatively by oxidizing these alcohols to their corresponding acids and titrating the acid mixture with alkali. Now this acid mixture, after having been titrated and thus having served the purpose of quantitative estimation of the fusel oil, was again acidified with sulphuric acid and submitted to fractional distillation. As was expected, the rate of distillation differed in the case of acid mixtures obtained from the fusel oils of different samples of liquors, such differences being obviously due to differences in the composition of the acid mixtures, hence also of the fusel oils in these samples. In other words, the fusel oil of a distilled liquor yields upon oxidation an acid mixture which, when fractionally distilled from an aqueous solution under definite conditions, passes over at a rate which is dependent upon the composition of the fusel oil. The curve representing that rate of distillation affords, therefore, a ready means to differentiate between fusel oils of different composition without making an actual analysis of those complex mixtures.

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[1] Presented by A. F. Seeker.

[2] See method of M. Duclaux of detectiog and estimating organic acids. Allen's Commercial Organic Analysis, 1905, 1: 490.

The esters of distilled liquors may also be studied qualitatively by isolating the sum total of their combined acid radicles and submitting the acid mixture to fractional distillation.

The procedure was in all cases as follows:

To 119 cc. of the acid mixture in a 500 cc. distillation flask, 1 cc. of concentrated H_2SO_4 was added, the flask connected to a condenser, and the solution distilled at a moderate rate, the receiver being changed each time 20 cc. passed over. Five fractions of 20 cc. each were distilled over, 20 cc. remaining in the distillation flask. Each fraction was titrated with standard alkali, thus furnishing a set of five numbers, representing the rate of distillation of the mixed acids, from which a curve graphically showing this rate of distillation could be estimated. The numbers were expressed as percentages of acid in each fraction with regard to the total acid that had passed over in the entire 100 cc. distilled. Thus, a rate of distillation represented by the following five numbers: 40, 25, 15, 10, 10, would mean that the first 20 cc. fraction contained 40% of total acid that distilled over in all the five fractions taken together, the second 20 cc. fraction contained only 25%, the third, 15%, the fourth, 10%, and the fifth, 10%.

Tables 1, 2, and 3 represent the results of fractional distillation of acid mixtures resulting from the oxidation of fusel oils derived from various distilled liquors.

TABLE 1.

Fractional distillation of fusel oil—French brandies.

SAMPLE NO.	PER CENT OF ACID IN EACH FRACTION					SAMPLE NO.	PER CENT OF ACID IN EACH FRACTION				
	1	2	3	4	5		1	2	3	4	5
1	41.0	25.9	15.6	9.6	7.8	20	36.2	23.2	15.2	12.4	13.0
2	41.0	24.8	15.4	10.2	8.5	21	38.6	23.9	14.5	11.0	12.0
3	40.6	22.6	15.1	10.9	10.8	22	38.2	23.1	15.6	11.6	11.5
4	40.7	23.2	13.9	11.1	11.1	23	40.6	24.1	14.4	10.7	10.2
5	43.8	21.9	14.4	9.6	10.3	24	41.9	23.9	14.4	10.5	9.3
6	38.3	23.4	17.0	10.7	10.6	25	38.0	23.0	15.6	11.8	11.6
7	42.4	24.3	14.4	10.8	8.1	26	38.3	24.0	15.3	11.2	11.2
8	40.2	23.9	15.2	10.9	9.8	27	40.1	22.9	14.4	10.6	11.9
9	42.7	24.3	15.5	8.8	8.7	28	40.1	23.2	15.0	10.6	11.1
10	45.7	23.1	13.0	9.2	8.9	29	41.2	22.4	13.7	10.7	12.0
11	39.4	24.8	15.2	10.4	10.2	30	40.3	24.3	14.8	10.4	10.2
12	38.9	23.6	15.4	10.9	11.2	31	41.8	24.6	15.6	9.8	8.2
13	41.1	24.3	14.8	10.0	9.8	32	41.9	25.0	14.5	10.2	8.4
14	41.5	22.9	14.6	10.6	10.4	33	38.5	24.8	13.7	11.2	11.8
15	45.5	23.5	13.4	9.0	8.6	34	36.0	23.6	15.6	12.4	12.4
16	46.6	23.3	13.5	8.2	8.4	35	45.2	25.2	14.0	8.3	7.3
17	36.0	23.4	15.1	12.0	13.5	36	41.2	24.3	15.0	10.5	9.0
18	38.0	23.2	15.1	11.4	12.3	37	39.1	24.2	15.2	11.4	10.1
19	38.4	23.5	14.4	11.6	12.1	38	37.3	23.7	15.9	11.9	11.2

Table 4 is a study, on identical lines, of the fractional distillation of acid mixtures representing the acid radicles of the esters. In the last case those acid mixtures were isolated in the following manner: The free acidity of the liquor having been determined in a separate portion, 100 cc. of it was neutralized exactly with the required amount of standard alkali and distilled. The distillate was saponified in the ordinary manner (as in the determination of esters) and again distilled till all alcohol had passed over. The residue in the distillation flask being then diluted with water, acidified with H_2SO_4 and distilled, the residue in the distillation flask was again diluted and again distilled. The combined distillates containing the volatile acid radicles of the esters were submitted to fractional distillation in the manner described above for fusel oil.

TABLE 2.

Fractional distillation of fusel oil—miscellaneous brandies.

SAMPLE NO.	PER CENT OF ACID IN EACH FRACTION				
	1	2	3	4	5
French brandy					
39	40.8	23.4	15.1	10.6	10.1
40	41.3	24.0	14.9	10.3	9.5
41	40.8	24.1	15.4	10.5	9.2
42	40.3	24.6	15.3	10.9	8.9
43	40.4	25.7	15.5	10.6	7.8
44	40.2	23.9	15.0	10.8	10.1
45	38.9	24.0	14.9	11.1	11.1
46	41.7	24.2	14.6	10.4	9.1
47	40.0	24.2	15.3	11.0	9.5
48	35.0	23.5	16.5	12.5	12.5
49	47.2	24.6	13.3	8.3	6.6
50	41.0	23.9	14.9	10.4	9.8
51	44.5	25.3	14.6	8.9	6.7
52	42.3	24.0	14.7	10.0	9.0
53	40.2	25.1	15.9	10.5	8.3
54	35.9	22.5	15.9	13.1	12.6
55	44.5	25.4	15.0	8.7	6.4
56	38.8	23.6	15.2	11.9	10.5
57	42.9	25.2	14.1	9.8	8.0
58	37.9	24.2	15.5	11.8	10.6
59	35.9	23.3	16.8	12.6	11.4
60	42.7	24.4	14.5	9.8	8.6
61	36.0	24.0	16.7	12.0	11.3
62	47.6	25.5	13.7	7.4	5.8
63	38.3	24.7	15.5	11.4	10.1
Hungarian brandy					
1	37.8	18.9	16.2	13.5	13.5
2	35.4	21.0	14.8	13.7	15.1
3	34.7	22.2	15.3	13.9	13.9
4	41.6	23.4	14.7	10.5	9.8
5	38.7	22.9	15.0	11.7	11.7
Italian brandy					
1	48.5	24.2	12.6	7.7	7.0
Greek brandy					
1	38.1	22.0	14.8	11.9	13.2
2	46.4	23.7	12.4	8.3	9.2
3	48.8	24.1	12.7	7.5	6.9
4	34.8	22.8	15.4	13.1	13.9
5	47.8	23.8	12.3	7.8	8.3
6	48.0	23.8	14.5	7.7	6.0

Of all the samples examined, only the eight samples marked "Authentic cognac" are known to be genuine cognacs. All the other samples are marked according to the legend on the label, and their history, origin, and mode of manufacture are not known. Handicapped as we are because of this uncertainty as to the origin and peculiarities in the methods of manufacture of almost all of the samples under examination, still it is possible to draw some general conclusions from a study of the tables submitted.

TABLE 3.

Fractional distillation of fusel oil—miscellaneous liquors.

SAMPLE	PER CENT OF ACID IN EACH FRACTION				
	1	2	3	4	5
Spanish brandy:					
No. 1	36.7	22.0	15.3	13.0	13.0
No. 2	40.4	22.9	15.3	10.7	10.7
Chile brandy	41.3	24.0	14.6	10.4	9.7
Batavia arrac	27.7	19.7	16.2	16.8	19.6
German plum brandy	36.3	23.1	15.6	12.5	12.5
Slivowitz	41.3	23.6	14.8	10.7	9.6
Pomace brandy (French):					
No. 1	36.6	21.7	14.7	12.6	14.4
No. 2	29.7	20.4	15.9	15.3	18.7
No. 3	29.9	19.6	15.7	15.6	19.2
Pomace brandy (Italian)	30.9	21.4	16.4	14.6	16.7
Jamaica rum	24.1	20.7	19.3	17.9	18.0
West Indies rum	46.9	24.1	12.7	8.2	8.1
St. Croix rum	47.0	23.5	12.8	8.4	8.3
Cuba rum	35.8	22.7	16.1	13.1	12.3
French rum	41.3	23.8	14.8	10.1	10.0
Raisin brandy:					
No. 1	46.6	25.7	13.5	8.1	6.1
No. 2	39.2	23.7	14.4	11.4	11.3
Brandy:					
No. 1	35.7	22.3	15.9	12.7	13.4
No. 2	29.4	19.6	16.3	15.7	18.9
No. 3	28.0	20.2	16.9	15.2	19.7
No. 4	46.4	25.4	13.6	8.2	6.4
Authentic cognac:					
No. 1	46.6	24.8	13.0	8.1	7.5
No. 2	44.3	23.1	13.5	9.4	9.7
No. 3	45.1	24.5	13.1	8.9	8.4
No. 4	49.7	25.2	12.4	7.0	5.7
No. 5	48.5	24.5	12.4	7.6	7.0
No. 6	46.6	24.4	13.0	8.4	7.6
No. 7	49.6	23.8	12.2	7.4	7.0
No. 8	45.2	24.1	13.7	8.5	8.5
Commercial fusel oil	49.9	24.6	12.0	7.0	6.5

(1) The combined acids of the esters in distilled liquors seem to be surprisingly constant in their composition, and their rate of distillation does not, therefore, offer as promising a means for detecting sophistication as is undoubtedly the case with the higher alcohols.

(2) In the case of the acids obtained by oxidation of the higher alcohols of brandies, the greatest variations are shown by the values for the first and fifth fractions, their averages being for—

	Per cent	Per cent
Authentic cognacs	47.0	7.7
French brandies	40.5	9.9
Pomace brandies	31.8	17.2

The values of the fourth fraction are usually not much different from those of the fifth fraction, and the variations in the values of the second and third fractions are rather small.

(3) The rate of fractional distillation seems to offer a promising means of differentiating between cognacs, ordinary brandies, and pomace brandies, and generally between distilled liquors where difference in geographical origin or mode of manufacture has affected the composition of the fusel oil complex.

TABLE 4.

Fractional distillation of esters—miscellaneous liquors.

SAMPLE	PER CENT OF ACID IN EACH FRACTION				
	1	2	3	4	5
French brandy:					
No. 1	18.3	16.1	18.0	20.7	27.0
No. 2	17.0	16.2	18.2	21.0	27.5
No. 3	21.2	15.9	17.1	20.5	25.2
No. 4	17.4	17.8	17.8	20.4	26.6
No. 5	16.7	16.8	18.3	21.3	26.9
No. 6	17.0	17.0	18.1	21.3	26.6
No. 7	18.1	16.1	18.0	20.6	27.2
No. 8	16.9	16.0	16.5	21.4	29.2
No. 9	16.6	16.2	18.2	21.2	27.8
No. 10	17.0	16.3	18.1	20.7	27.9
No. 11	18.8	16.3	18.0	19.8	27.1
No. 12	16.6	16.4	18.2	21.1	27.7
No. 13	17.6	16.3	17.2	21.1	27.8
No. 14	17.3	16.5	18.4	20.8	27.0
No. 15	17.3	16.9	18.5	21.3	26.0
No. 16	17.5	16.5	18.1	21.2	26.7
Authentic cognac:					
No. 1	21.0	17.3	17.3	19.4	25.0
No. 2	19.4	16.4	17.9	20.2	26.1
No. 3	20.3	17.0	18.0	19.8	24.9
No. 4	20.9	17.7	18.0	19.3	24.2
Pomace brandy:					
No. 1	19.4	17.0	17.6	20.7	25.3
No. 2	17.6	16.7	18.1	21.1	26.5
No. 3	19.0	16.6	18.6	20.0	25.8
Greek brandy:					
No. 1	23.4	16.7	17.5	18.5	23.9
No. 2	18.2	16.1	18.4	20.9	26.4
No. 3	16.0	16.0	18.0	20.9	29.1
No. 4	16.6	13.3	19.4	22.0	28.7
Rum (Jamaica)	19.2	17.3	17.9	20.3	25.3
Rum (French):					
No. 1	20.4	15.8	17.3	20.6	25.9
No. 2	18.4	16.7	18.1	21.1	25.7
Rum (Martinique)	19.6	16.7	18.1	19.2	26.4
Rum (St. Croix)	16.4	16.4	17.5	21.2	28.5
Rum (West Indies)	15.7	16.7	18.5	21.8	27.3
Apricot liquor	18.9	16.8	18.8	20.4	25.1
Batavia arrac	15.9	16.7	18.2	21.1	28.1

(4) In one case—that of French brandy (No. 17, Table 1)—which gave upon analysis rather high figures for fusel oil and esters, thus apparently indicating good quality, suspicion as to its genuineness was aroused by the fact that the fractional distillation of the acids from the fusel oil gave the following figures: 36.0, 23.4, 15.1, 12.1, 13.5, indicative of

pomace rather than of ordinary brandy. The sample was thereupon submitted to two expert tasters, both of whom agreed in pronouncing the sample sophisticated. There are a number of brandies marked "French brandies" in Tables 1 and 2 whose fractional distillation curves are more or less similar to the curve mentioned above, and thus point the same way; that is, that they are not genuine brandies.

(5) A comparative organoleptic test of the "Authentic cognacs" seems to point to the fact that the finer the flavor and aroma of the cognac the higher the value of the first fraction in the fractional distillation.

(6) To sum up: The results so far obtained warrant the belief that valuable information relative to the origin and character of distilled liquors can be gained by ascertaining the rate of distillation of the mixed acids obtained by oxidation of their higher alcohols. In order to establish the value and limitations of the method, it will be necessary to work upon a sufficiently large number of samples from various sources the history of which is well authenticated.

APPLICATION OF THE PROCEDURE TO THE ANALYSIS OF A FLAVORING ESSENCE.

Table 5 shows the rate of distillation of formic, acetic, propionic, butyric, and valeric acids and also of simple mixtures of some of these acids when distilled under conditions indicated above in the discussion of fusel oils in distilled liquors.

The figures in the table are averages of two determinations in each case except in the case of formic acid, where an average was taken of four determinations. The separate determinations ordinarily check within 0.5%, but formic acid behaves in a rather erratic manner, its figures varying sometimes as much as 2%. Column 6 of Table 5 gives the percentage relation of the total acid in the total distillate of 100 cc. to the acid originally present in the distillation flask.

It is obvious that use may be made of the fractional distillation for the following purposes:

(1) Identification of any of the acids under discussion when present in a state of purity.

(2) Isolation of any of the acids in a pure state from a mixture of two and sometimes more acids for the purpose of identification.

(3) Testing for purity of any of the above acids.

(4) Analysis of a simple mixture of two and sometimes even more of the acids.

The above is the basis of M. Duclaux's method (as quoted in Allen's Commercial Organic Analysis) of identification and estimation of the lower acids of the acetic acid series.[1] In this connection it is well to note that

[1] See also article by A. Landolt, Unterscheidung naturlicher und kunstlicher Fruchtather, in Chem. Ztg., 1911, 35: 677.

the composition of an acid mixture cannot always be computed from its fractional distillation curve with any satisfactory degree of accuracy, as the acids mutually affect each other during distillation. The curve of the mixture nevertheless gives an approximate idea of its composition, and two or three experiments are generally sufficient to make up a known mixture of the given acids that will yield upon fractional distillation a curve practically identical with the one from the unknown mixture. The total acid in both the known and unknown mixture should be approximately the same in order that the results of their fractional distillation might be comparable, as the fractional distillation curve is somewhat affected by the concentration of the acid to be distilled. The limits of permissible variation in the strength of the acid to be distilled without affecting the distillation curve have not been determined, though, as will be seen from Table 5, a 100% variation in the strength of an approximately N/100 acid does not materially affect its distillation curve.

TABLE 5.

Fractional distillation of acids.

ACID IN DISTILLATION FLASK	MIXTURES	PER CENT OF ACID IN EACH FRACTION					TOTAL ACID IN COMBINED DISTILLATE
		1	2	3	4	5	
cc. N/10							*per cent*
8.5–17.7	Formic acid............	11.6	13.9	17.2	22.0	35.3	42.8
11.6– 7.5	Acetic acid.............	14.8	17.1	18.9	21.8	27.4	68.1
6.4–12.8	Propionic acid.........	24.5	22.6	20.7	17.8	14.4	92.1
7.3–15.6	Butyric acid[1]..........	36.1	27.1	18.4	11.8	6.6	94.0
11	Valeric acid[2]...........	54.6	27.2	12.2	4.3	1.7	97.0
30	2 molecules valeric plus 1 molecule acetic......	45.6	24.8	13.5	8.5	7.6	87.5
21	2 molecules valeric plus 1 molecule propionic..	44.7	25.9	15.0	8.8	5.6	96
21	2 molecules valeric plus ½ molecule acetic plus ½ molecule formic.....	46.5	24.8	13.4	8.1	7.1	84
58	1 molecule valeric (14.5 cc.) plus 3 molecules acetic (43.5 cc.).......	28.2	20.5	16.8	16.1	18.4	....
....	1 molecule valeric (11.6 cc.) plus 4 molecules acetic (46.4 cc.)........	25.8	20.0	17.2	17.1	19.9	....
48	1 molecule valeric (8 cc.) plus 5 molecules acetic (40 cc.).........	24.4	19.4	17.4	17.7	21.1	....
49	1 molecule valeric (7 cc.) plus 6 molecules acetic (42 cc.).........	23.0	19.2	17.6	18.4	21.8	....

[1] Of uncertain purity.

[2] Obtained by oxidation of amyl alcohol (Kahlbaum's).

A complete analysis of a flavoring essence—a so-called banana oil—has been made in this laboratory by a procedure based largely on the above-described facts and considerations involving the fractional distillation of acids and their mixtures. A detailed description of the procedure followed will now be given as an illustration of the possibilities of the method.

DESCRIPTION OF METHOD.

About 5 grams of the banana oil was saponified with an excess of aqueous KOH by boiling under reflux condenser. The total alcohols in the saponification product were then distilled off and thus two solutions obtained:

(1) The residue in the distillation flask containing the total combined acids from the esters, together with a small amount of acid originally free in the oil and which has been determined in a separate portion of the oil by direct titration; and

(2) The alcoholic distillate containing the total alcohol, free and combined, of the original oil.

The excess of alkali in solution (1) was titrated with sulphuric acid and the total combined and free acid in the oil thus estimated. The neutralized solution was then acidified and an aliquot fractionally distilled. The values obtained for the fractions were as follows: 22.7, 19.1, 17.7, 18.8, 21.7. Ratio of acid in total distillate to acid in distillation flask, 72%.

With a view of isolating the component acids of the mixture, fraction 1 and also the residue in the distillation flask were, upon proper dilution, again fractionally distilled. The latter (the residue in distillation flask) yielded values for the fractions practically identical with those of pure acetic acid. The first fraction, however, yielded a curve indicating a mixture and not a simple acid. The first fraction in this, the second distillation, was again refractionated and the operation repeated until after the fifth fractional distillation of the first fractions values were obtained practically indentical with those of a pure valeric acid. Thus was the acid mixture in the saponification product proved to be a mixture of valeric and acetic acids. It was then established by direct experiment that a mixture of one molecule valerie and six molecules acetic acids would yield upon fractional distillation the following values: 23.0, 19.2, 17.6, 18.4, 21.8. Ratio of acids in total distillate to acid in distillation flask, 71.7%.

The above values are practically identical with those obtained from the acid mixture in the saponification product of the banana oil. Thus was the composition of the total acid (free and combined) in the banana oil determined to be as follows:

	cc. N/10 per gram
Free acid (assumed to be acetic)	2.0
Total combined acids (by saponification)	67.4
Combined acetic acid (by fractional distillation)	57.5
Combined valeric acid (by fractional distillation)	9.9

An aliquot of the solution of the total acids was neutralized, evaporated off, dried, and weighed, and the weight agreed fairly well with the composition indicated above.

The alcoholic distillate from the saponification product containing the total alcohol (free and combined as asters) of the banana oil was oxidized with alkaline permanganate to their corresponding acids.[1] The resulting acids were distilled off, the total acidity of the distillate titrated, and the solution then examined for its component acids qualitatively and quantitatively by a system of fractional distillation, exactly in the same manner as outlined above for the combined and free acids in the oil.

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[1] See method of C. R. Smith and A. S. Mitchell, U. S. Bur. Chem. Bul. 122: "Determination of Fusel Oil by Alkaline Permanganate."

The acid mixture resulting from the oxidation of the total alcohols (free and combined) in the oil proved to be composed as follows:

	cc. N/10 per gram
Valeric acid	55
Acetic acid	30

thus indicating that the total alcohols were composed as follows:

	cc. N/10 per gram
Amyl alcohol	55
Ethyl alcohol	30

The above results were checked in the following manner:

The original banana oil was saponified with alcoholic potash, all the alcohol in the saponification product distilled off, and the alcoholic distillate examined for higher alcohols in exactly the same manner as fusel oil is determined in brandy (Allan Marquart fusel-oil method). Titration of the final acid distillate gave 55 cc. N/10 acid per 1 gram oil, and the fractional distillation of the solution gave values for the fractions identical with those of pure valeric acid.

Further examination of the banana oil gave the following data:

Free ethyl alcohol was proved to be absent; the presence of amyl acetate, ethyl acetate, and amyl valeriate was indicated by the odor of various fractions obtained from a fractional distillation of the original banana oil; nonvolatile unsaponifiable matter, 1%.

Summing up the results obtained, we arrive at the following composition:

	cc. N/10 per gram
Free acid (as acetic)	2.0
Combined acetic acid	57.5
Combined valeric acid	9.9
Combined ethyl alcohol	30.0
Combined amyl alcohol	37.4
Free amyl alcohol	17.6
Nonvolatile unsaponifiable (per cent)	1

and the rational formula may be expressed as follows:

	cc. N/10 per gram	per cent
Free acid (as acetic)	2.0	1.2
Amyl valeriate	9.9	17.0
Ethyl acetate	30.0	26.4
Amyl acetate	27.5	35.7
Free amyl alcohol	17.6	15.5
Nonvolatile unsaponifiable		1.0
		96.8

No report was made by the associate referee on vinegar.

REPORT ON SPICES.

By Harry E. Sindall (Philadelphia, Pa.), *Associate Referee.*

The work done this year falls under two headings—moisture determinations on whole spices and the total ash of herbs.

MOISTURE.

No recommendations were made by last year's associate referee for further work on spices, but it seemed desirable this year to study a method for determining moisture in whole spices. No samples were sent out, as the nature of the work rendered it unsuitable for coöperative investigation. The work was done on whole cloves and whole black pepper. The method under consideration was the Brown-Duvel distillation method for moisture in grain. But since unsatisfactory results were obtained by using Renown engine oil, or in fact any engine oil, the best grade kerosene oil was substituted and found to give more uniform results.

The method as worked by the associate referee is as follows:

> Place 50 grams of whole spice in a distilling flask with 150 cc. kerosene, whirl the flask several times to bring the oil in contact with each particle of spice. Place the flask on an asbestos board. Cut so that the bottom of the flask extends below the surface. A wire gauze with an asbestos center is placed about one-half of an inch below the bottom of the flask. The object is not to bring the flame in direct contact with the flask, and the asbestos board serves to keep the heat uniform. Connect the flask directly with a vertical condenser and collect the distillate in a graduated cylinder or burette. Insert a thermometer through the stopper of the distilling flask, extending down into the oil. Adjust the flame so that about twenty minutes will be required to reach the temperature of 170°C. Now extinguish the flame, after which the thermometer will show a slight gradual increase in temperature. As soon as the water stops dropping from the condenser tube, which usually requires from four to six minutes, the operation is complete. Multiply the volume of the water layer by 2 to obtain the percentage of moisture.

The following table shows results obtained by drying 2 grams of whole cloves and whole black pepper at 110° C. to constant weight; from the resulting loss in weight the amount of volatile ether extract obtained by extracting the whole spices was subtracted.

The samples marked (a) were taken before the cloves went on the mill to be ground; the samples marked (b) represent the same cloves immediately after grinding. The loss according to weight by grinding was 6.26%.

Comparative results on moisture.

	BY EXTRACTION	BY DISTILLATION		BY EXTRACTION	BY DISTILLATION
	per cent	*per cent*		*per cent*	*per cent*
Whole cloves ..	(a)13.94	(a)11.2	Ground cloves.	10.16	....
Do........	15.98	12.6	Whole pepper..	6.02	6.0
Do........	11.24	9.8	Do........	7.95	6.8
Do........	10.61	9.5	Ground pepper.	7.87	8.0
Ground cloves.	(b) 6.11	(b) 6.0			

This loss consists chiefly of moisture and some volatile oil, due to the fact that the cloves are more or less heated while passing through a high-speed mill.

The large percentage of moisture in the cloves as shown in the above table under extraction method seems due to the fact that all the volatile oil was not extracted by the ether, as shown in the following table:

Volatile ether extract.

	per cent		*per cent*
Whole cloves	2.88	Ground cloves	15.74
Do	2.89	Whole pepper	0.30
Do	3.13	Do	0.53
Do	3.31	Ground pepper	2.13
Ground cloves	13.91		

TOTAL ASH DETERMINATION.

The associate referee's attention was called to wide discrepancies in the results of total ash determinations obtained by different chemists on samples taken from the same lot of herbs. For the purpose of studying this matter, large samples of marjoram, sage, savory, and thyme were ground under the associate referee's supervision, and subdivisions of these were sent to collaborators, with instructions to determine the total ash in each sample by the method commonly used by the respective collaborators and describe the method used. Reports were received from eight collaborators:

Total ash.

ANALYST	MARJORAM	SAGE	SAVORY	THYME
	per cent	*per cent*	*per cent*	*per cent*
F. L. Shannon, Lansing, Mich.	(a) 14.34	(a) 9.265	(a) 13.335	(a) 12.89
	(b) 14.135	(b) 9.275	(b) 13.29	(b) 12.78
C. L. Black, Philadelphia, Pa.	16.16	9.45	14.06	13.14
H. B. Mead, Philadelphia, Pa.	15.84	9.75	14.05	13.42
C. S. Brinton, Philadelphia, Pa.	15.21	9.39	13.63	13.47
	15.10	9.43	13.72	13.21
A. P. Coulture, Ottawa, Canada.	14.54	9.47	13.68	12.47
	14.61	9.50	13.56	12.59
Paul Rudnick, Chicago, Ill.	13.42	9.31	12.60	13.14
	13.44	11.21	12.68	12.48
C. O. Dodge, Washington, D. C.	14.05	8.41	12.41	13.46
J. H. Bornmann, Chicago, Ill.	14.08	9.80	13.77	13.67
	14.48	9.82	13.85	13.43

Mr. Dodge also determined nonvolatile matter and ash on a nonvolatile basis, with the following results:

	NONVOLATILE MATTER	ASH ON NON-VOLATILE BASIS
	per cent	*per cent*
Marjoram	92.6	15.17
Sage	93.0	9.05
Savory	91.6	13.54
Thyme	92.9	14.48

METHODS OF COLLABORATORS.

Mr. Shannon made the determinations in duplicate. Sample (a) in each case was ignited in an ordinary platinum milk dish; sample (b) in a fused silica dish of the same size. All were burned first over a Meker burner at a very low red heat and then exposed to a low red heat in a muffle, cooled in a desiccator, and weighed.

Mr. Black used 2-gram portions in platinum dishes which were held about one-fourth inch above bottom of muffle by means of asbestos board supported by small pieces of pipestem. The bottom of the muffle was kept at a barely visible red heat. After ashing was apparently complete, the residue was moistened with alcohol to reveal any unburned carbon and then reheated if necessary until treatment showed no unburned carbon.

Mr. Mead used 2-gram samples in flat-bottom round dishes, dimensions about 3 inches by 1 inch. These were elevated on pieces of pipestems about three-eighths inch above the bottom of the gas muffle heated to a visible red. The dishes were below visible red. The ash was moistened with alcohol and reheated. There was practically no carbon or change after the first heating.

Mr. Brinton used 2-gram portions and made the combustion in small platinum dishes about 1 inch square and approximately three-eighths inch deep. At no time during any of the work were the dishes heated to a red heat. The determinations were made in a gas-heated muffle, the bottom of the muffle at times being more or less red; but the dishes were supported on pieces of asbestos board about one-fourth inch or more above the bottom of the muffle. An air space, therefore, separated the asbestos board from the red-hot bottom of the muffle. The asbestos board was supported by pieces of crucible lid.

Mr. Coulture used 2-gram samples, and charred them in platinum at a heat below redness. This temperature was found to be sufficient to completely burn off all the carbon. He did not use a muffle. After weighing, dish and contents were heated again slightly below red heat for a period of half an hour and the operation repeated to constant weight. After a time there was a slight increase in weight, and the minimum was taken as being the true weight of the ash.

Mr. Rudnick used 2 grams in a small flat-bottom porcelain dish and ignited carefully at a low temperature so as to avoid loss of sample. The ignition was continued at a low red heat and finally at a bright red for a few minutes, then cooled and weighed. The appearance of the ash after weighing was carefully noted to be sure that all carbon had been burned off, also to note whether particles of sand and foreign matter other than true ash were apparent.

Mr. Dodge followed the method given in U. S. Bureau of Chemistry Bulletin 107 (revised), except that the products were dried at 110°C. for four hours and weighed, then dried for one hour and again weighed. The greatest loss during the second drying was less than 1%.

Mr. Bornmann charred 2 grams of the well-mixed substance in a platinum dish over asbestos, using a small flame. The carbon was burned off in a muffle at a temperature below redness.

CONCLUSIONS.

These results are not encouraging. While the samples submitted were known to contain several per cent of sand, it is not believed that the discrepancies can possibly be due entirely to this cause, but that they were attributable, in part at least, to the temperature employed in making the combustion. It would seem, at all events, that the subject warrants further investigation, particularly along the line of operating at definite temperatures.

RECOMMENDATIONS.

It is recommended—

(1) That the associate referee's modification of the distillation method for water in spices be given further study.

(2) That the subject of ash determination in herbs be further studied, with particular reference to the influence of the exact temperature employed in the combustion.

NOTE REGARDING THE DETERMINATION OF CRUDE FIBER IN BLACK PEPPER.

By A. E. Paul (Bureau of Chemistry Food and Drug Inspection Laboratory, Chicago, Ill.).

Two samples, one with a low fiber content and the other high in this constituent, were sent to various laboratories in this district, with the request that analyses be made. The results follow:

Analyses of black pepper.

SAMPLE NO. 1.

ANALYST	NON-VOLATILE ETHER EXTRACT	VOLATILE ETHER EXTRACT	TOTAL ASH	ASH INSOLUBLE IN HCl	CRUDE FIBER	PENTOSANS
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
J. H. Bornmann, Chicago	8.03	0.70	5.09	0.57	10.46	....
	8.27	0.83	5.41	0.61	11.38	....
E. H. Berry, Chicago	....	....	....	....	11.09	....
J. Feldbaum, Chicago	8.10	1.04	5.35	0.62	11.08	....
H. D. Grigsby, Cincinnati	8.19	0.55	5.24	0.51	11.99	....
J. S. McCune, St. Louis	8.87	....	5.16	0.43	10.41	6.1
C. L. Clay, New Orleans	8.69	....	4.62	0.34	11.26	6.0
SAMPLE NO. 2.						
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
J. H. Bornmann, Chicago	7.20	1.03	6.48	0.95	16.60	....
	7.26	1.01	6.61	1.00	17.11	....
E. H. Berry, Chicago	....	....	....	....	16.60	....
J. Feldbaum, Chicago	7.15	1.18	6.48	0.92	16.80	....
H. D. Grigsby, Cincinnati	7.18	0.57	6.43	0.79	17.41	....
J. S. McCune, St. Louis	7.82	....	6.37	0.76	15.84	8.40
C. L. Clay, New Orleans	7.80	....	5.48	0.56	17.23	7.90

In view of the very great discrepancy in the various fiber determinations, a new 5-pound sample was taken by an analyst, carefully mixed, quartered to a small bulk, and transferred to a small sample bottle.

The remainder was again carefully mixed, and a small subsample withdrawn as before. Then still a third sample was taken in the same manner. The analyst was requested to make crude-fiber determinations in duplicate on each subdivision.

The remainder of the pepper was then delivered to another analyst, who prepared three subsamples in the same manner as above.

Both analysts were instructed to stir the material in the bottle very carefully with a spoon of approximately 2-grams capacity, to withdraw a spoonful from the interior of the bottle, and carefully transfer the entire contents to the balance pans. It was then left to the option of the analysts to utilize the quantity thus taken for the fiber determination, or else to remove carefully the slight excess over 2 grams, or add sufficient to make that amount. Of the two analysts, Mr. Berry preferred the former detail, while Mr. Bornmann chose to work on the exact 2 grams. Their results were:

ANALYST	SAMPLE NO. 1	SAMPLE NO. 2	SAMPLE NO. 3
	per cent	*per cent*	*per cent*
E. H. Berry	13.36	12.98	13.46
	13.15	13.00	13.16
J. H. Bornmann	13.03	13.15	13.25
	13.25	13.07	13.31

These results are quite acceptable, and show that the trouble is not in the method itself, but rather in the manner of mixing and weighing out the samples.

PRELIMINARY REPORT ON DETERMINATION OF ASH, ESPECIALLY IN SPICES.

By Clement S. Brinton (Food and Drug Inspection Laboratory, U. S. Appraiser's Stores, Philadelphia, Pa.)

The object of this paper is to emphasize the necessity of carefully following the directions for the determination of ash as given in the Official and Provisional Methods of Analysis of this association (U. S. Bur. Chem. Bul. 107, rev.), particularly where, under "Ash, official" for the analysis of foods and feeding stuffs, the following language is used: "* * * burn until free of carbon at the lowest possible heat" (p. 38). Again, under "Preparation of ash," etc., (p. 238), it is directed to "Conduct the combustion at a comparatively low temperature, never employing a full red heat * * *." Other similar directions could be cited.

The desirability of preparing the material mentioned herein arose through trouble in obtaining closely agreeing results for ash by different analysts during the examination of spices, especially those having normally a high sand-free ash, such as marjoram with 11 to 16% of ash and thyme with from 10 to 14%.

This lack of agreement was found to be due not to faulty sampling but to lack of uniformity in the analytical method. This was ascertained by having portions of a large sample of marjoram finely ground, about 60 mesh, thoroughly mixed, and analyzed in seven laboratories in the Eastern States according to the procedure usually employed in those laboratories. Determinations were made in platinum dishes in all cases except laboratory 6, where a porcelain crucible was used. Electric muffles were used in laboratories 1 and 4, and probably in laboratory 5; gas muffles were used in laboratories 2, 3, and 7; and laboratory 6 burned in open flame. The results submitted are given in Table 1.

TABLE 1.

Ash determinations on marjoram—first report by collaborators.

LABORATORY	ANALYST	TOTAL ASH	REMARKS
		per cent	
1	W. C. T.	15.21 15.24	Muffle below redness; time of burning, 1 hour. Not observed carefully to ascertain when free of carbon.
		15.16 15.18	Time of burning, 4 hours additional; otherwise same as above.
2	W. J. McG.	13.47 13.45	Muffle high red; time of burning not stated. Heated to constant weight.
3	J. B. L. J. E.	15.14 14.90	Muffle low cherry red; time of burning not stated. Ash stirred with rod from time to time until carbon burned. Analyst J. E. heated longer and found no loss in weight.
4	F. O. W.	15.11 15.13 15.11 15.09	Muffle below redness; time of burning not stated. Moistened with water to observe presence of carbon; again heated and weighed when free of carbon.
5	L. P.	15.40 15.30	Muffle below redness; time of burning not stated. Moistened with water after weighing and found free of carbon.
6	H. E. S.	13.24 13.35	Open flame with crucible bright red; time of burning, 40 minutes. Ash determined as he usually does in spices.
7	C. L. B.	15.68 15.70	Dish not visibly red; time of burning not stated. Ash treated with water after weighing to test for carbon.
	H. B. M.	16.11 16.16 16.03 15.69	Bottom of muffle below visible red heat; time of burning not stated. Moistened and reheated before weighing; weighed after free of carbon.

The writer obtained results ranging from a maximum of 15.8% down to a minimum of 14.89%, according to conditions, all results being obtained when the dish was not at a visible red heat as viewed in the open muffle by daylight. All determinations were made in platinum dishes about 1 inch square, one-half inch deep, in a 5-burner gas Wiesnegg muffle. The results are given in Table 2.

TABLE 2.

Ash determinations on marjoram—results by author.

DATE (1915)	TOTAL ASH		REMARKS
	Dish 1	Dish 2	
	per cent	*per cent*	
May 24	15.43	15.46	Time of burning, 1¾ hours in all. Dishes on bottom of muffle entire time. Heated 1½ hours below visible red heat, then raised to dull red for one-fourth hour.
	15.01	14.89	Muffle just visible red on bottom; time of burning, 35 minutes. Dishes on bottom of muffle entire time and carbon-free when moistened with alcohol.
May 25	15.37	15.75	Not a visible red; time of burning, 35 minutes. Both dishes about one-fourth inch above bottom of muffle on crucible lids. Muffle open. Dish with high result in front of other.
May 26	15.74	15.86	Not a visible red; time of burning, about 2½ hours. These results from same conditions as above except muffle not so hot. After 1½ hours tested but not carbon-free. After weighing and getting these results found carbon free.
June 3	15.06	15.39	Bottom dull red in spots; time of burning, about 2 hours. One dish in front of other; otherwise same as second set May 24. Dishes raised off bottom and not a visible red.
	15.20	15.48	Bottom dull red in spots; time of burning, only 35 minutes in all. Carbon all burned out. One dish in front of other. Dishes raised off bottom.
June 8	15.18	15.10	Bottom dull red in spots; time of burning, only 37 minutes in all. Carbon all burned out. Dishes side by side and supported one-fourth inch above bottom on asbestos board. Neither asbestos board nor dishes a visible red. Hot air could circulate under and over asbestos and dishes.
June 9	15.70	15.65	Time of burning, about 6 hours. Conditions same as on June 8, but lower temperature used. Both ashes moistened after weighing and free of carbon. Dishes side by side.

TABLE 2.—*Continued.*

DATE (1915)	TOTAL ASH		REMARKS
	Dish 1	Dish 2	
	per cent	*per cent*	
June 9	23.35	23.87	Muffle slightly red in spots; time of burning, 17 minutes. Much carbon present. Conditions same as preceding.
	16.18	16.20	Muffle slightly red in spots; time of burning, 10 minutes long. Carbon still present in both.
	15.88	15.68	Dishes not visible red; time of burning, additional 10 minutes. Traces of carbon in both; most in higher result.
	[1]16.37	[1]16.10	Dishes not visible red. Both moistened, evaporated to dryness, heated in muffle below redness before weighing.
	16.15	15.93	Dishes not visible red. Same as preceding after one-half hour longer in muffle.
	15.75	15.65	Dishes not visible red. Same as preceding after 1 hour longer in muffle; temperature slightly higher.

[1] Note the effect of moistening with water and subsequent long heating required to drive off this water and bring result down to that obtained before moistening.

The results reported on May 26 agreed closely with those obtained by Analyst C. L. B., but are slightly below those reported by Analyst H. B. M. Further attempts to duplicate the latter's results were in vain, as shown by work subsequent to May 26. This later work brought out very emphatically the fact that this sample of marjoram contained mineral matter very susceptible to slight changes in temperature, length of time of burning, etc., and that without exact duplication of temperature and other conditions closely agreeing results at different times could not be determined. See various results in Table 2 on June 3, June 8, June 9, and June 10, where determinations in duplicate in every particular, except that one dish was in front of the other and in slightly cooler part of muffle, show variations of 0.3%, and yet both are free of carbon.

It was found that only when the two dishes were placed side by side could close duplicates be obtained, as shown by results of June 8 and June 9, each set of which agrees closely; but there is a marked difference of about 0.5% because of different temperatures, the dishes in neither set being a visible red, although the bottom of muffle was dull red in spots. The second set of results on June 9 were made at a low temperature and weighed at short intervals to determine if some easily volatile mineral matter was present which was completely volatilized about the time the carbon was completely burned. The tabulated results show that lower results were obtained than reported by Analyst H. B. M., yet some carbon was still present. Just at this point, the writer learned that Analyst H. B. M. had moistened his ash before weighing to uncover unburned carbon, then added enough alcohol to make a mixture which would burn, ignited the alcohol and burned it off, and replaced the dish in muffle for a short time, then cooled and weighed as usual. Because of this analyst obtaining higher results than anyone else and higher than the writer could obtain in any other way, the last set in Table 2 when about free of carbon were moistened, treated with alcohol, etc., about the same as did Analyst H. B. M., and it was found that his high results as originally reported could be duplicated, but in no other way; therefore, the conclusion was made that the water treatment before weighing explained his higher results. Later work by the author confirms this conclusion (see Table 4). The work reported in Table 2 convinced the author that for marjoram especially, and probably in other substances high in ash, an accurate determination of the ash required that more care be given to the control of the temperature than is usually given, and it was thought that higher results would be reported by the various analysts if their attention was called to this point. Consequently, the following method was sent to the previous collaborators:

> Take 2 grams of the well-mixed sample in a platinum dish and burn at *lowest possible temperature*, below a visible red heat, until free from carbon when examined by the eye; cool, and weigh as usual. Now moisten ash with 95% alcohol, and examine for small specks of carbon. If specks of carbon are found, ignite alcohol, allow to burn off, and then return dish to muffle and allow to heat a little longer at same temperature. Cool and weigh again, repeating this operation until all carbon is burned off.

All results obtained were requested, also an opinion as to the true value for ash in the sample of marjoram sent out, the various results previously submitted by all of them being sent also so they would see the reason for further work. The second set of results are given in Table 3.

TABLE 3.

Ash determinations on marjoram—second report by collaborators.

LABORATORY	ANALYST	TOTAL ASH		REMARKS
		Dish 1	Dish 2	
		per cent	*per cent*	
1	H. R. S.[1]....	15.47	....	Time of burning not given. Two grams in flat-bottom platinum dish, center cold electric muffle; rheostat set for temperature below red heat and burned till free of carbon.
		15.56	....	Same as above, except that when entirely charred dish was withdrawn. Ash covered with alcohol, ignited, and again heated in muffle *below red heat*. After 2 treatments with alcohol carbon-free.

[1] General comment by analyst; "It is evident that prolonged heating at even very low temperature does cause a loss of inorganic matter by volatilization. Also the most practical and reliable method for determining ash in organic products is that outlined in method 3 above."

NOTE.—No report was received from laboratories 6 and 7.

TABLE 3.—*Continued.*

LABORATORY	ANALYST	TOTAL ASH		REMARKS
		Dish 1	Dish 2	
		per cent	*per cent*	
1	H. R. S.	16.00	....	Same as above at start. When charred, removed, cooled, and all soluble ash leached out with hot water and filtered in usual manner. Filter and char burned in dish below red heat. Solution of soluble ash added, evaporated to dryness on steam bath, and finally heated at low temperature and weighed.
		15.04	....	Same as above at start. Rheostat set for lowest possible temperature; allowed to heat all night (16 hours).
2	W. J. McG.	15.10	....	Time of burning not given. Heated in gas muffle just below red heat till carbon had disappeared, and after one alcohol treatment. After heating at red heat for 30 minutes, obtained 13.03%, 13.06%, and a further drop to 12.46%, 12.54% after 30 minutes more at red heat.
		15.18		
		15.24		
		14.96		
3	J. F. D.	16.16	16.46	Visible carbon present.
		15.74	15.87	Do.
		15.63	15.77	Do.
		15.37	15.76	Visible carbon absent.
		15.36	15.77	Total time of burning, 9 hours. Constant weight.
				Six treatments with alcohol were required. Muffle at all times below visible red heat. These results are about 0.5% higher than previously reported from this laboratory, but analyst does not consider increase in accuracy justifies increase of time.
4	F. O. W.	15.03	....	Time of burning not given. "The heating was done very slowly at a low temperature with proper precautions until no specks of carbon were left."
		15.06		
		14.95		
5	L. P.	15.66	15.65	Time of burning not given. Burned in electric muffle at lowest temperature possible. When carbon seemed all gone, weighed, then moistened with alcohol and found carbon-free. Temperature is slightly lower than used for first results.
		14.91	....	Time of burning not given. Obtained at higher temperature than above, but still a temperature which shows no redness in muffle or in dish.
		14.69	....	Time of burning not given. Obtained on another determination by higher heat than preceding. Analyst considers last two results worthless.

Comments on Table 3 by author: All laboratories submitting reports obtained higher results by using lower temperatures, etc., than first reported, with the single exception of laboratory 4. Further, these higher results, with two exceptions, agree very well with those obtained in laboratory 7, and those of the author as given in Table 2. The results of Analyst H. R. S., where he used water to moisten ash, confirm those of Analyst H. B. M. and the author, where water was added. The tenacious combination of this water is remarkable since, as shown by the author in Table 2, about 1½ hours' heating at approximately 400°C. was required to drive it off and bring the ash down to the same value as before its addition.

The author, about this time, suggested similar work to the associate referee on spices. He has already submitted his results.

TABLE 4.

Ash determinations on marjoram and stramonium with temperature control.

MARJORAM.

DATE (1915)	TOTAL ASH	MAXIMUM TEMPERATURE BY PYROMETER	TIME BURNING	ASH AFTER MOISTENING AND REHEATING	TIME REHEATING	MAXIMUM TEMPERATURE WHILE REHEATING	INCREASE DUE TO MOISTENING	REMARKS
	per cent	*°C*	*h. m.*	*per cent*	*h. m.*	*°C.*	*per cent*	
Oct. 8...	15.69	(1)	(1)	15.94	1	400	0.25	Muffle entirely closed except 1-inch hole in front and three-eighths inch hole in back.
	15.90	400	(1)	16.20	1	400	0.30	Do.
Oct. 12..	15.25	(2)	1 35	15.43	1 51	400	0.18	Quartz dish. Muffle wide open. An increase of about 0.2 % after almost 2 hours heating.

STRAMONIUM.

DATE (1915)	TOTAL ASH	MAXIMUM TEMPERATURE BY PYROMETER	TIME BURNING	ASH AFTER MOISTENING AND REHEATING	TIME REHEATING	MAXIMUM TEMPERATURE WHILE REHEATING	INCREASE DUE TO MOISTENING	REMARKS
	per cent	*°C.*	*h. m.*	*per cent*	*h. m.*	*°C.*	*per cent*	
Oct. 26..	18.21	400–440	4 30	18.45	1 50	400	0.24	
	18.27	400–440	4 30	18.37	1 50	400	0.10	

[1] Not noted.

[2] 440°C. during combustion of carbon, then constant at 400°C.

The data given in Table 4 show results obtained where a pyrometer was used in the open gas muffle. The majority of these results are not satisfactory because of difficulties in maintaining a constant temperature in the muffle. The temperature and results reported are only approximate, but show very plainly that where an accurate result is desired on a substance like marjoram, great care must be observed to have exactly identical conditions, or considerable variations must be expected. The effect of moistening the ash is again plainly brought out in this table.

The drug stramonium has also a high ash, and some little work has been done on one sample (see Table 4).

This paper is a preliminary one intended to emphasize certain phases of the determination of ash in spices, especially the necessity of using a low temperature, *below redness at lowest possible temperature*, as specified in the Official and Provisional Methods of Analysis (p. 162) previously cited. The writer expects to present more data later, together with conclusions as to the composition of the mineral matter which volatilizes so easily at or below redness. Evidence will also be presented to show that with products like marjoram, thyme, etc., it is better to determine ash in an open muffle, allowing plenty of circulation of air, instead of in a closed muffle, where, owing to an absence of oxygen, a longer time is required to burn the carbon.

CONCLUSIONS.

(1) The mineral matter in marjoram, thyme, and other plant products having a high ash is easily volatile to a very marked degree at a temperature just below redness.

(2) Great care is necessary by analysts in ashing these products. Temperatures below visible redness must be used or erroneous and low results will be obtained.

(3) After moistening the ash with water the weight before moistening is not regained at the temperature of combustion, even after a reheating of over one hour.

(4) Further work on this subject is necessary so that the official methods will plainly caution analysts against the troubles mentioned above.

The committee for the suggestion of changes in the constitution and by-laws, previously authorized by the association, was announced as follows: B. B. Ross, chairman; C. L. Alsberg, and H. D. Haskins.

REPORT ON FLAVORING EXTRACTS.

By A. E. Paul (Bureau of Chemistry Food and Drug Inspection Laboratory, Chicago, Ill.), *Associate Referee.*[1]

Three methods were studied last year by collaborators on flavoring extracts:

(1) The saponification method for wintergreen extract, devised by Hortvet and West.

(2) The brine method for anise and nutmeg extracts, as described by Hortvet and West.

(3) The Howard-Mitchell method, slightly modified, for peppermint, spearmint, and wintergreen extracts.

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[1] Presented by E. K. Nelson.

In each case the method was recommended for further study by the association. Inasmuch as the results reported last year appeared to be quite satisfactory, these three methods only were submitted this year, in the hope that the results obtained would be such as to warrant their final adoption as provisional in this meeting.

Of each extract two samples were submitted, one of full standard strength, as directed in U. S. Bureau of Chemistry Circular 19, and the other one-fourth that strength. The nutmeg extracts, therefore, contained 2.0% and 0.5% of oil, while all the others contained 3.0% and 0.75%. All were made with 95% alcohol, which gives the test of the methods the maximum of severity. In the case of the weaker extracts this was perhaps more severe than necessary, since the lower grades of extracts are usually made with more or less dilute alcohol.

SAPONIFICATION METHOD FOR WINTERGREEN EXTRACT.

In connection with the saponification method for wintergreen extract, Mr. C. B. Gnadinger suggested titrating instead of weighing the separated salicylic acid. Details for this operation were added to the method as previously studied, and collaborators were requested to express an opinion as to their preference. It seems that the same idea had occurred to other operators, and that essentially the same details have already been in use in other laboratories. The results reported and the comments submitted follow:

Analyses of wintergreen extract.

COLLABORATORS	OIL PRESENT	OIL FOUND			
		Gravimetric		Volumetric	
	per cent	*per cent*		*per cent*	
C. O. Dodge	0.75	0.78		0.73	
	3.00	3.01		2.90	
C. B. Gnadinger	0.75	0.76	0.76	0.75	0.75
	3.00	2.99	2.98	2.94	2.94
C. F. Jablonski	0.75	0.74	0.74	0.73	0.73
	3.00	2.96	2.97	2.93	2.94
H. L. Lourie	0.75	....		0.79	0.77
	3.00	....		2.98	2.98
J. P. Street	0.75	0.80	0.74	....	
	3.00	3.01	2.97	....	
C. F. Sutton	0.75	0.76	0.74	0.76	0.74
	3.00	2.99	2.99	2.98	2.98
A. R. Todd	0.75	0.90		0.80	
	3.00	3.06		2.97	
P. B. Yost	0.75	0.77	0.80	0.78	
	3.00	3.02	3.05	3.06	
Averages	0.75	0.77		0.76	
	3.00	3.00		2.96	

COMMENTS BY COLLABORATORS.

C. B. Gnadinger: Method very satisfactory. Little choice between gravimetric and volumetric modifications. I prefer the latter because it is slightly shorter.

Chas. F. Jablonski: Suggest combining the two methods by titrating the weighed residue.

C. E. Morrison (J. P. Street): The salicylic acid was determined gravimetrically, which method is preferred in this laboratory.

Clarence F. Sutton: Titrated the weighed salicylic acid.

A. R. Todd: The method appears to be very satisfactory. It seems to me that the amount of water used in transferring the solution to a separatory funnel could be cut down to 15 cc.

P. B. Yost: I prefer the original method of weighing, as I find end point in the titration to be rather indefinite.

Analyses of anise and nutmeg extracts.

COLLABORATORS	ANISE			NUTMEG		
	Oil present	Oil found		Oil present	Oil found	
	per cent	*per cent*		*per cent*	*per cent*	
E. H. Berry	0.75	0.8	0.8	0.5	0.5	0.5
	3.00	3.2	3.2	2.0	2.0	2.0
C. L. Black	0.75	0.75	0.8	0.5	0.5	0.6
	3.00	3.1	3.1	2.0	2.0	2.0
E. Bloomberg	0.75	(1)	(1)	0.5	(1)	(1)
	3.00	3.4	3.4	2.0	2.0	2.0
Jacob Feldbaum	0.75	0.8	0.8	0.5	0.6	0.5
	3.00	3.0	3.2	2.0	1.8	2.0
C. B. Gnadinger	0.75	0.7	0.7	0.5	0.5	0.4
	3.00	3.0	2.9	2.0	1.9	1.9
E. J. Munch	0.75	1.0		0.5	0.8	
	3.00	3.2		2.0	2.2	
L. Patton	0.75	(1)	(1)	0.5	(1)	(1)
	3.00	3.4	3.4	2.0	2.0	2.0
E. W. Thornton	0.75	0.9		0.5	0.2	
	3.00	3.2		2.0	2.0	
G. W. Trainor	0.75	1.0	1.0	0.5	0.8	0.8
	3.00	3.2	3.0	2.0	2.2	2.1
Averages	0.75	0.83		0.5	0.56	
	3.00	3.2		2.0	2.01	

[1] See comments.

COMMENTS BY COLLABORATORS.

C. L. Black: I found it necessary to use C. P. NaCl, and that even nicer oil layers are obtained by using half saturated sodium sulphate solution.

L. Patton and E. Bloomberg: Stated that the oily layer formed in the case of the dilute extracts did not cover the brine, and that, therefore, an exact reading was not possible; that, however, the amount of oil present is more than a mere trace.

C. B. Gnadinger: Without being advised as to the amount of oil actually present, the results appear to be excellent. If they are reasonably correct, I believe the method should be made official.

E. W. Thornton: The separation in each case was satisfactory without centrifuging a third time.

Analyses of spearmint, peppermint, and wintergreen extracts.

COLLABORATORS	OIL PRESENT	OIL FOUND		
		Spearmint	Peppermint	Wintergreen
E. H. Berry	0.75	0.8	0.8	0.8
	3.00	3.0	3.0	3.1
R. D. Cook	0.75	0.9	0.9	0.8
	3.00	3.0	3.1	2.7
C. B. Gnadinger	0.75	0.7 0.8	0.8 0.8	0.8 0.8
	3.00	2.8 2.9	3.0 2.9	3.0 3.0
J. S. McCune	0.75	0.7 0.8	0.8 0.8	0.7 0.7
	3.00	3.0 3.0	3.0 3.0	3.0 2.8
E. J. Munch	0.75	0.8 0.8	0.6 0.6	0.8 0.8
	3.00	2.8 2.9	3.0 3.0	3.0 3.0
K. J. Osterhout	0.75	0.7 0.8	0.8 0.8	0.8 0.8
	3.00	2.8 2.7	3.0 3.0	3.0 3.0
G. W. Trainor	0.75	0.9 1.0	0.9 0.9	0.9 0.9
	3.00	2.8 3.0	3.0 3.0	2.9 2.9
Averages	0.75	0.8	0.8	0.8
	3.00	2.9	3.0	2.9

COMMENTS BY COLLABORATORS.

E. H. Berry: This method worked very satisfactorily, as it did last year. It certainly should be made official.

C. B. Gnadinger: I think the method is an excellent one, and should be made official.

K. J. Osterhout: Thinks that there may be some volatilization of oil in the case of spearmint.

CONCLUSIONS.

The results throughout are quite satisfactory. The saponification method for wintergreen yielded results which are truly remarkable in accuracy and in concordance. As to the details for determining the amount of salicylic acid separated, there seems to be very little choice between the gravimetric and volumetric procedure. The gravimetric results appear to be a little closer, and it would seem preferable to retain the method in its original form.

In the brine method for anise and nutmegs, the results for the standard extracts are excellent. Even for the dilute preparations, the reports are very satisfactory.

The carbon bisulphid method for peppermint, spearmint, and wintergreen yielded most satisfactory results.

Referring particularly to wintergreen extract, it would seem desirable to adopt both the saponification method and the bisulphid method, for the reason that the former gives the percentage of actual methyl salicylate present, while the latter gives the percentage of oil. The presence of a foreign oil, therefore, will become apparent if both methods are used. It is suggested, however, that the saponification method be described as a method for methyl salicylate.

RECOMMENDATIONS.

In view of the above results, supplementing and corroborating the outcome of last year's study on flavoring extracts, the following recommendations are respectfully submitted:

(1) That the saponification method of Hortvet and West for methyl salicylate in wintergreen extract, as described in the Journal of Industrial and Engineering Chemistry, 1909, No. 1, and slightly modified in U. S. Bureau of Chemistry Bulletin 152, page 141, by the then associate referee, R. S. Hiltner, be adopted as provisional. The method follows:

Mix 10 cc. extract in a 100 cc. beaker with 10 cc. of potassium hydroxid solution (10%). Heat on a boiling water bath until volume is reduced about one-half. Add a distinct excess of dilute hydrochloric acid, cool, and extract with three portions of ether, 40 cc., 30 cc., and 20 cc., respectively. Filter the combined ether extracts through a dry filter into a weighed dish, wash with 10 cc. ether, and evaporate spontaneously. Dry over calcium chloride in a desiccator and weigh. The weight of salicylic acid thus obtained multiplied by 9.33 gives the percentage of oil of wintergreen by volume.

(2) That the following method, devised by Hortvet and West, and described in the Journal of Industrial and Engineering Chemistry, volume 1, No. 1, be made provisional for anise and nutmeg extracts:

To 10 cc. extract in a Babcock milk flask add 1 cc. of hydrochloric acid (1 : 1), then sufficient half-saturated salt solution previously heated to 60°C. to fill the flask nearly to the neck. Cork and let stand in water at 60°C. for about 15 minutes, occasionally giving the flask a twisting motion, and centrifuge for 10 minutes at about 800 revolutions per minute. Add brine till the oil rises into the neck of the bottle, and again centrifuge for ten minutes. If the separation is not satisfactory, or the liquid is not clear, cool to about 10°C. and centrifuge for an additional ten minutes. Multiply the reading by 2 to obtain the percentage of oil by volume.

(3) That the following slight modification of the Howard-Mitchell method, which has been studied during the last two years, be now provisionally adopted for peppermint and spearmint extracts and for the determination of oil in wintergreen extract. The method follows:

Pipette 10 cc. of the extract into a Babcock milk bottle, add 1 cc. of carbon disulphid, mix thoroughly, then add 25 cc. of cold water and 1 cc. concentrated hydrochloric acid. Close the mouth of the bottle with the thumb and shake vigorously, whirl the bottle in a centrifuge for six minutes, and remove all but 3 or 4 cc. of the supernatant liquid, which should be practically clear, by means of a glass tube of small bore, and aspiration.

Connect the stem of the bottle with a filter pump, immerse the bottle in water kept at approximately 70°C. for three minutes, removing from the bath every 15 seconds and shaking vigorously. Continue in the same manner for 45 seconds, using a boiling water bath. Remove from the bath and shake while cooling.

Disconnnect from the suction and fill the bottle to the neck with saturated salt solution at room temperature, centrifuge for two minutes, and read the volume of the separated oil from the top of the meniscus. Multiply the reading by 2 to obtain the percentage of oil by volume.

In the case of wintergreen, use as floating medium a mixture of 1 volume of concentrated sulphuric acid and 3 volumes of saturated sodium solution.

Since submitting the above report on flavoring extracts, two further collaborators submitted their reports. The first was by A. G. Woodman, of Boston, whose results by the methods submitted are similar to those obtained by the other collaborators, but he makes a very interesting statement regarding a nephelometric method which he has tried on very dilute extracts, and he reported his results on the dilute samples of anise and nutmeg submitted. His results were:

	OIL PRESENT	OIL FOUND
	per cent	*per cent*
Anise	0.75	1.00
Nutmeg	0.50	0.50

While Mr. Woodman's details were not available, a few simple tests were made along the line suggested, and it is believed that the idea is promising and that when Mr. Woodman's paper is available it may be desirable to study the method for use on very dilute preparations.

The second paper received was by C. O. Dodge, of the Bureau of Chemistry in Washington. His results on the wintergreen extracts by the saponification method, slightly modified by himself, are highly accurate and are as follows:

	OIL PRESENT	OIL FOUND
	per cent	*per cent*
Wintergreen extracts:		
No. 1	0.75	0.75
No. 2	3.00	2.99

Mr. Dodge suggests that the details submitted may be improved by keeping down the bulk of the solution to be extracted and using a smaller amount of ether. However, the details suggested by him involve four extractions with a total of 60 cc. of the solvent, while the original method requires only three extractions with a total of 90 cc. of ether. Inasmuch as the results obtained by the collaborators were very satisfactory, there is doubt that the changes are necessary.

Mr. Dodge raises the further point that oil of wintergreen may contain only 98% methyl salicylate, and suggests that the calculation of the result should be based on this figure. It seems, however, that this objection is overcome by the recommendation that this determination be considered a determination of methyl salicylate rather than a determination of oil.

REPORT ON BAKING POWDERS.

By H. E. Patten (Bureau of Chemistry, Washington, D. C.),
Associate Referee.

Tests of three gravimetric methods for the determination of lead in phosphate baking powders were made. Five analysts coöperated. The methods studied were the Seeker-Clayton, Remington modification of the Seeker-Clayton, and Exner methods. Directions for these methods were sent out as follows:

DESCRIPTION OF METHODS.

SEEKER-CLAYTON METHOD.

Place 20 grams of the baking powder in a large beaker and add about 25 cc. of water in small portions at a time to avoid excessive frothing. Add 20 cc. of concentrated hydrochloric acid, a little at a time for the same reason, and digest on a steam bath until the solution is perfectly clear and limpid or until a drop of the solution gives no reaction for starch with iodin and potassium iodid solution. Add sufficient solution of ammonium citrate[1] (lead-free) to correspond to 20 grams of citric acid and render slightly alkaline to litmus with ammonia, density 0.95. The latter is added a little at a time, with care to keep the solution cool, to avoid precipitation of calcium salts. Dilute to about 400–450 cc., add 10 cc. of 10% hydrochloric acid, cool to room temperature, saturate with hydrogen sulphid, and allow to stand overnight.

Filter through a moistened filter, using suction if necessary, and wash the precipitate[2] with hydrogen sulphid water and finally with a little water. Dissolve the precipitate by passing through the filter three 5 cc. portions of boiling 10% hydrochloric acid followed by three 5 cc. portions of boiling 25% nitric acid, and collect the filtrate in a 100–150 cc. beaker. Finally wash the filter with a little hot water, add 2 cc. of concentrated sulphuric acid to the filtrate and washings, and evaporate on a hot plate until fumes of sulphuric acid are copiously evolved. The solution should now be practically colorless, but if not so add a little nitric acid and again evaporate until fumes appear. Cool, add 10 cc. of water and 20 cc. of alcohol, and allow to stand overnight. Filter through a Gooch on asbestos and wash with alcohol.

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[1] *Ammonium citrate solution.*—This reagent may be prepared by dissolving 100 grams of citric acid in 100 cc. of hot water, cooling, adding a little at a time sufficient ammonia to leave a slight excess, again cooling, and then saturating with hydrogen sulphid. Allow to stand overnight, or until the sulphids have settled out, filter, boil the filtrate to expel excess of hydrogen sulphid and ammonia, cool, and make up to 200 cc. Lead-free citric acid may be used instead of this solution, but it has the disadvantage of causing a considerable evolution of heat in the subsequent neutralization with ammonia, resulting in a precipitate of calcium citrate.

[2] In some cases, especially when a very large amount of calcium phosphate is present, a white precipitate of calcium citrate will settle out on standing overnight. In such a case, decant the supernatant liquid through a filter and dissolve the precipitate in a small amount of dilute hydrochloric acid, add an excess of ammonium citrate, cool, render slightly alkaline with ammonia, cool, saturate with hydrogen sulphid, and allow to stand from 6 to 12 hours. The precipitated sulphids are then filtered off and treated as in the regular method. (The material remaining on the filter from the liquid first decanted has in the meantime been washed with hydrogen sulphid water and dissolved in hot hydrochloric and nitric acid, the solution being finally combined with that from the second crop of lead sulphid.)

Place the Gooch in a small beaker and treat the contents with a few drops of concentrated ammonia. Then pour 10 cc. of 50% ammonium acetate into the crucible and allow it to stand for about 15 minutes. Remove the crucible from the beaker and carefully wash the bottom and sides with water, allowing the washings to run into the beaker. Now, by placing the lips over the top of the crucible, blow the solution still remaining in the crucible into the beaker. Wash the crucible with a little water, forcing the washings through the asbestos pad in the manner just described. Rinse the bottom of the crucible with a jet of water and fit it into a bell jar arranged for filtering by suction. Filter the contents of the beaker through the Gooch, collecting the filtrate in a second beaker placed under the bell jar, and wash thoroughly with hot water. Acidify the filtrate with acetic acid, heat nearly to boiling, add an excess of potassium bichromate, and allow to stand overnight. Then filter through a tared Gooch, dry for 20–30 minutes on a hot plate, cool, and weigh as $PbCrO_4$.

REMINGTON MODIFICATION OF THE SEEKER-CLAYTON METHOD.

Weigh 100 grams of the baking powder into a large beaker (1.5–2 liters) and add 500 cc. of the cold citrate solution[1] slowly with stirring. If the powder contains albumin a troublesome frothing may result, which can be controlled by the use of a few cubic centimeters of ether. Stir thoroughly several times, and set aside for several hours.

Decant the supernatant liquid, and wash the starch twice by decantation with about 50 cc. of water, adding the washings to the original solution. (A centrifuge will be of great assistance in the decantation and washing.) Heat the solution on the steam bath until the albumin is coagulated, and pass in a slow stream of hydrogen sulphid for several hours, or overnight. (If a heavy white precipitate is formed, add ammonia cautiously until it is dissolved, taking care that the solution remains acid.) Filter off the precipitated sulphids and wash with water containing hydrogen sulphid.

To the residue of starch add 250 cc. of water and 25 cc. of hydrochloric acid, and heat on the steam bath until the starch is completely hydrolized. Neutralize with ammonia, and make just acid to litmus with hydrochloric acid. Pass in a slow stream of hydrogen sulphid for several hours, or overnight. Filter and wash with water containing hydrogen sulphid.

Place the filters containing both precipitates in a tall 100 cc. beaker, add 5 cc. of sulphuric acid and 10 cc. of nitric acid, and heat on the hot plate, with frequent additions of nitric acid until all organic matter is destroyed and the liquid is nearly colorless. Take down to fumes of sulphuric acid, cool, add 20 cc. of water and 40 cc. of alcohol, and allow to stand overnight.

From this point proceed as in the original Seeker-Clayton method, as follows:

Filter through a Gooch on asbestos, and wash with alcohol. Place the Gooch in the original beaker and moisten the contents with a few drops of concentrated ammonia. Then pour 10 cc. of 50% ammonium acetate solution (previously treated with H_2S and boiled) into the crucible and allow it to stand for about fifteen minutes. Remove the crucible from the beaker and carefully wash the bottom and sides with water, allowing the washings to run into the beaker. Now, by placing the lips over the top of the crucible, blow the solution still remaining in the crucible into the beaker. Wash the crucible with a little water, forcing the washings through the asbestos pad in the manner just described. Rinse the bottom of the crucible with a jet of water, and fit it into a bell glass fitted for filtering with suction. Pass the ammonium acetate solution through the Gooch, filtering twice if necessary to secure a perfectly clear filtrate, and wash thoroughly with a little hot water. Acidify the filtrate with acetic acid, heat nearly to boiling, add an excess of potassium dichromate, and allow to stand overnight. Filter on a small tared Gooch, wash the precipitated lead chromate with cold water, dry the crucible and contents by heating for 20–30 minutes on a hot plate, cool, and weigh as $PbCrO_4$.

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[1] *Preparation of ammonium citrate reagent.*—Dissolve 200 grams of ammonium citrate in a liter of water, add 10 cc. of concentrated hydrochloric acid, warm slightly, and pass in a slow stream of hydrogen sulphid for several hours. Filter and heat to boiling to expel excess of H_2S.

EXNER METHOD.

Weigh out 200 grams[1] of the sample and transfer this to a 3-liter Jena flask. Add 300 cc. concentrated nitric acid in portions, with thorough shaking after each addition. The mixture, which first forms a thick paste, is slowly heated on an asbestos gauze with repeated shaking. The mass becomes thinner, and by the time the oxidation of the starch begins it is quite fluid. As soon as brown fumes begin to appear at the mouth of the flask, heat is removed and a stemless funnel is inserted in the neck of the flask. The reaction soon becomes very vigorous, and the flask should be set in a hood with a good draft. The reaction, however, will not become so violent as to cause loss of material by foaming over. When the action has moderated, the flask is set back on the asbestos gauze over a moderate Bunsen flame. When the action becomes weak, as shown by the fumes in the flask becoming lighter, 90 cc. concentrated sulphuric acid is slowly added to the contents of the flask, and heating is again continued until the fumes fade. Then 25 cc. of concentrated nitric acid are added from time to time, with continued heating, until all the starch is completely oxidized. This can be told from the behavior of the last addition of nitric acid, which boils out with but little decomposition. Usually three or four additions of 25 cc. portions of nitric acid suffice. The nitric acid should finally be expelled as completely as possible without endangering the flask. Cool and add 400 cc. distilled water, shake, and allow to settle. The soluble sulphates of sodium, potassium, aluminum, iron, etc., go into solution, while calcium sulphate and most of the lead sulphate will be precipitated. Filter through an 18 cm. folded filter into a liter Erlenmeyer flask; rinse the 3-liter flask two or three times with small portions of water and pour the rinsings through the filter. There is no need of a thorough washing of the precipitate. The latter is transferred to a 2-liter Erlenmeyer flask. This is best done by opening the filter containing the precipitate over a 600 cc. beaker and rinsing thoroughly with water from a wash bottle. The contents of the beaker are then transferred to the Erlenmeyer, together with whatever precipitate remains in the 3-liter flask. The contents are then diluted so as to nearly fill the flask, stirred thoroughly to dissolve the calcium sulphate, 20 cc. strong acetic acid added, and the liquid thoroughly saturated with hydrogen sulphid. This may be done quickly by preparing a solution of sodium sulphid by saturating a 5% sodium hydroxid solution completely with hydrogen sulphid, drawing up this liquid into a pipette, which is then dipped into the solution in the Erlenmeyer and stirred while the sulphid runs out. Only a small part of the free acid need be neutralized to produce complete saturation with hydrogen sulphid. This procedure saves both time and gas. The flask is then corked and set aside until the precipitate has settled. The liquid is then siphoned off, saving much time in filtering. The lower end of the siphon tube which is introduced into the liquid has a short bend at right angles so that it will not suck the precipitate from the bottom. When there is much calcium sulphate, one such treatment will not suffice to bring all into solution, but the flask is refilled with distilled water, is again acidified and saturated with hydrogen sulphid and allowed to settle till the calcium sulphate is practically all dissolved and the residue of sulphids is dark colored. When much calcium sulphate is present, solution may be hastened by the addition of lead-free sodium acetate to the water, 50–75 grams to each 2 liters of water. If the solution of the salt gives no darkening with hydrogen sulphid, it may be safely used.

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[1] If other weights have to be used, take reagents in proportion.

The liquid containing the soluble sulphates is treated separately to recover the trace of lead which it may contain. The acidity is partially neutralized with ammonium hydroxid just short of the point of producing a permanent precipitate of aluminum phosphate. It is then saturated with hydrogen sulphid and the precipitate allowed to settle. Some iron sulphid will usually be precipitated also. It is important that the sulphid precipitations be made in very slightly acid solutions, otherwise lead sulphid will not be completely precipitated. When both precipitates have finally settled, the liquids may be siphoned off, and the precipitates transferred on to separate 11 cm. filters and washed with hydrogen sulphid water. The filters with their precipitates are placed into a 200 cc. Erlenmeyer flask, 10 cc. concentrated nitric acid and 5 cc. concentrated sulphuric acid are added, a stemless funnel inserted in the neck, and the flask heated to completely oxidize the material. When the nitric acid has all been expelled and the residue darkens, more nitric acid is added until no such darkening occurs. The residue is finally heated till fumes of sulphur trioxid are given off. Then cool and add 15 cc. of water. Filter through 7 cm. filter, rinse, and then wash the filter twice with small portions of dilute sulphuric acid, and finally with a little water. There is no need of removing all sulphuric acid. Place a clean 150 cc. beaker under the filter, dissolve the precipitate with 15–25 cc. ammonium acetate and wash thoroughly with water. The ammonium acetate is best prepared by mixing 1 part 99% acetic acid, 1 part water, and 1 part ammonium hydroxid sp. gr. 0.90, introducing a piece of litmus paper, and cautiously adding more ammonium hydroxid until neutral.

The solution of lead in ammonium acetate is acidified with acetic acid and precipitated with potassium bichromate. The mixture is heated on the steam bath and then allowed to cool and settle. It is filtered on to a tared Gooch having a thick felt, washed with water, dried at about 125°C., and weighed as lead chromate.

It is taken as a matter of course that the water used and the reagents must be free from lead. There seems to be no danger from lead in vessels when Jena glass is used.

SAMPLE SOLUTIONS.

In view of the lack of confidence expressed by many analysts with whom we consulted in the Seeker-Clayton method as originally given, it was decided to send out for a preliminary test samples containing the lead in a soluble form. A very slight amount of organic material was added to necessitate wet oxidation as recommended by the methods. Some of the collaborators very properly objected on the ground that these samples did not offer the same analytical difficulties met with in the analysis of phosphate baking powders. They have, nevertheless, worked faithfully upon the samples and have sent in data which, in the judgment of the associate referee, fully justify the style of sample used for this preliminary test of the comparative merits of the methods. Further, to eliminate personal equations and any autosuggestion in the analysts, three sample solutions labeled A, B, and C were sent out, each containing exactly the same concentration of lead (as $Pb(NO_3)_2$), but without indicating to the analysts that these solutions were duplicates one of the other so far as the lead was concerned. The ingredients of the solutions are given below.

It will be noted that the diluted solutions made from these samples which were finally to be taken for the lead tests contained a quantity of lead the same as would be found in a baking powder containing 20 parts of lead per million. Thus, 200 grams of baking powder (Exner method) containing 20 parts per million of lead would have 4 mg. of lead. One hundred grams (Remington method) would contain 2 mg. of lead, and 20 grams (Seeker-Clayton method) would contain 0.4 mg. The solutions measured out for analysis contained these respective amounts.

INGREDIENTS OF SOLUTIONS.

Solution A contained 10 grams of sugar per 100 cc. and 40 mg. of lead per 100 cc. (as $Pb(NO_3)_2$), with a few drops of concentrated nitric acid to repress hydrolysis.

Solution B was an exact duplicate of solution A and was taken from the same mix.

Solution C was identical with solutions A and B except that 2.5 grams $CaH_4 (PO_4)_2 H_2O$ (found to be lead free) was added per 100 cc.

DIRECTIONS ACCOMPANYING SAMPLE SOLUTIONS.

The following directions were sent out with the solutions:

> Take 50 cc. of each solution at 25°C. and make up with distilled water to 500 cc. at 25°C. Then for use in the Exner method use 100 cc. of this diluted solution; for the Remington method use 50 cc.; and for the Seeker-Clayton method use 10 cc. The results should be reported in milligrams per liter of original solution.

REPORTS OF COLLABORATORS.

The collaborators reported results which are tabulated as milligrams per liter of original sample solution in Table 1. These results have been recalculated to a basis of parts per million of a phosphate baking powder, using the weights of baking powder recommended for analysis in each of the methods, respectively, and are given in Table 2.

One of the collaborators, Dr. T. J. Bryan, in addition to the requested work, added to the diluted solutions to be taken for analysis an amount of lead-free baking powder required by the different methods and then

TABLE 1.

Lead in milligrams per liter of original solution as reported by the collaborators.

[Solutions contained 400 mg. of lead per liter.]

COLLABORATOR AND ANALYST	LEAD IN MILLIGRAMS PER LITER OF SOLUTION								
	Solution A			Solution B			Solution C		
	Seeker-Clayton	Remington	Exner	Seeker-Clayton	Remington	Exner	Seeker-Clayton	Remington	Exner
Loomis (Wichman)....	128	333	333	128	307	358	128	422	416
Hortvet (Pettijohn)..	1220	640	870	640	500	370	380	460	420
	2750	990	......	700	380	......	450	210	......
	......	......	......	700	......	......	......	......	......
Morey (Hoyt)........	260	550	359	380	360	436	260	396	378
	380	475	372	320	422	429	190	410	359
Bryan...............	384	371	454	833	358	211	1020	269	358
	897	294	371	512	179	237	1020	294	397
Stallings (Clarke)....	513	436	442	513	410	410	448	576	481
	448	422	416	448	422	384	513	512	512
	385	......	......	......	448	......	577	......	......
	......	......	......	......	422	......	......	......	......

TABLE 2.

Recalculation of results given in Table 1 to a basis[1] *of parts per million in a phosphate baking powder.*

[Solutions contained 20 parts of lead per million.]

COLLABORATOR AND ANALYST	LEAD IN PARTS PER MILLION								
	Solution A			Solution B			Solution C		
	Seeker-Clayton	Remington	Exner	Seeker-Clayton	Remington	Exner	Seeker-Clayton	Remington	Exner
Loomis (Wichman)....	6	17	17	6	15	18	6	21	21
Hortvet (Pettijohn)..	61	32	44	32	25	19	19	23	21
	138	50	......	35	19	......	22	11	......
	......	......	......	35	......	......	......	......	......
Morey (Hoyt)........	13	28	18	19	18	22	13	20	19
	19	24	19	16	21	21	10	21	18
Bryan...............	19	19	23	42	18	11	51	13	18
	45	15	19	26	9	12	51	15	19
Stallings (Clarke)....	26	22	22	26	21	21	22	29	24
	22	21	21	22	21	19	26	26	26
	19	......	......	......	22	......	29	......	......
	......	......	......	......	21	......	......	......	......

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[1] For synthesis of samples see page 218.

TABLE 3.

Lead found in solutions to which lead-free baking powder was added in the proportions required by the various methods.

[Solutions contained 400 mg. of lead per liter.]

METHOD	LEAD IN MILLIGRAMS PER LITER OF SOLUTION		
	Solution A	Solution B	Solution C
Seeker-Clayton..........................	704	320	384
	448	192	192
Remington...............................	410	179	102
	397	38	140
Exner....................................	134	121	313
	281	294	

subjected the solutions to analysis. These results are shown in Table 3. Table 4 contains the results given in Table 3 recalculated to parts per million of lead in a phosphate baking powder in order to make them comparable with similar results given in Table 2.

TABLE 4.

Recalculation of results of Table 3 to a basis of parts per million in the phosphate baking powder added.

[Solutions contained 20 parts of lead per million.]

METHOD	LEAD IN PARTS PER MILLION		
	Solution A	Solution B	Solution C
Seeker-Clayton	35	16	19
	22	10	10
Remington	22	9	5
	20	2	7
Exner	7	6	16
	14	15	

DISCUSSION OF RESULTS.

Recurring now to the objections which have been made by some of the collaborators (H. M. Loomis, T. J. Bryan, C. B. Morey): They were of the opinion that these methods for the estimation of lead should be used upon lead-free baking-powder samples to which known quantities of lead had been added, or upon sample solutions containing the same quantity and ratio of ingredients that would occur upon solution of a phosphate baking powder in acid.

These methods have two objects: One, to determine the lead quantitatively, and the other to separate the lead from the mixture in which it is found. It seemed advisable to study first their respective merits in determining lead under conditions where there was practically no interference from baking-powder ingredients. If a method can not stand this test, manifestly there is no use of trying it out where there is added the further difficulty of separating the lead from a complex mixture of ingredients.

The reason that the associate referee sent out these very simple samples for analysis was to ascertain if these three methods could give comparable results in the hands of capable analysts under the most simple conditions. If this had proved to be the case, it was then the intention to carry out further work upon more complicated samples fully simulating the actual conditions found in phosphate baking powders. Owing to the lateness of the date at which the present associate referee took up this work, it has been possible to proceed only as far as the report indicates.

In the work done by Mr. Pettijohn, under Dr. Hortvet, the prescribed methods were not followed exactly in all cases; and for this reason, together with the wide variation in results presented, it seems fair to judge the methods on the results reported by the other four analysts.

SEEKER-CLAYTON METHOD.

VARIATIONS REPORTED.

The Seeker-Clayton method might possibly be adapted for this work by substituting a colorimetric reading at the end instead of the present gravimetric determination, but in its present form it gives results varying from (Table 2) 6 parts per million to 51 parts per million, using one solution with four different analysts, and from 19 parts per million to 51 parts per million for one analyst on three solutions (Bryan).

WEIGHING LIMITATION ERROR.

The inherent difficulty with the Seeker-Clayton method is the small amount of sample taken. With the baking-powder standard for lead set at 20 parts per million upper limit, there will be 0.0004 gram of lead in a 20-gram sample containing this highest limit. Since the lead is weighed as $PbCrO_4$, the actual mass weighed would be 0.000624 gram. Assuming that the balance weighs accurately 0.0001 gram, then any weight between 0.00055 gram and 0.00065 gram will be read as 0.0006 gram of $PbCrO_4$. This weight calculated to lead gives 0.000385 gram lead, with a range from 0.000353 gram to 0.000417. Calculating to parts per million of lead, 0.0006 gram $PbCrO_4$ represents 19.25 parts per million, and the range is 3.2 parts per million (from 17.65 to 20.85). Hence we have a possible error of ±8.3% inherent in the limitations of the balance when estimating lead in baking powders containing approximately 20 parts per million, even when the balance weighs accurately to 0.0001 gram. The above standard referred to (20 parts per million of lead) is for baking-powder acid-ingredient chemicals. The standard for complete baking powders is one-half of this, or 10 parts per million. A baking powder containing this upper limit would yield only 0.0003 gram $PbCrO_4$ and, the weighing errors remaining the same, the percentage of error due to the limitations of the balance would be doubled.

WEIGHT STANDARDIZATION ERROR.

The above discussion of weighing errors assumes that the set of metric weights used with the balance is rigidly standardized, and that each correction of standardization is used throughout all operations. To illustrate how great such an error may be, test 11388, U. S. Bureau of Standards, of a new set of weights (Becker's Sons, Rotterdam) showed the following: One of the 2-gram weights was too heavy by 0.1 mg., each 100-mg. weight was 0.04 mg. too light, and the 50-mg. weight was 0.02

mg. too light. Thus, a weighing in which one of the 2-gram weights was replaced by the other would show 0.0001 gram more or less than the correct mass. Similarly, a weighing in which the two 100-mg. weights and the 50-mg. weights were used together would lack 0.0001 gram of the true mass. Thus the possible error through neglecting the standardization of weights would be as large as the above mentioned error due to the limitations of the balance. And even when weight standardization is taken care of, constant use and corrosion of weights may introduce error.

OPTIMUM LOAD.

In making such close estimates as required by the Seeker-Clayton method, the effect of the balance load on the sensibility must be taken into account. It would, of course, be advisable to determine the load of greatest sensibility, and then add weights to the pan containing the Gooch crucible until this optimum load is approximated; or a lighter crucible might be chosen in case the optimum load were lower.

DRYING ERROR.

The method of drying the $PbCrO_4$ precipitate on the hot plate according to the Seeker-Clayton directions has been found to be subject to an error of 0.0002 gram by Dr. T. J. Bryan in his report, although he does not specify exact reasons for this error. Mr. Pettijohn also has criticized this procedure, but without quantitative expression. This error may be partially due to absorption of moisture during the weighing or in the balance case.

RECOMMENDATIONS ON SEEKER-CLAYTON METHOD.

The Seeker-Clayton method, because of the small quantity of sample and reagents required, may well serve as a semiquantitative method in the hands of one skilled in its use; but taking into account all of the factors mentioned, it would seem inadvisable to recommend the Seeker-Clayton method for further study.

REMINGTON MODIFICATION.

VARIATIONS REPORTED.

The Remington modification method showed much closer checking than the Seeker-Clayton, the greatest variation being from 13 to 29 parts per million with one solution and four different analysts, and from 9 to 19 parts per million with three solutions for one analyst (Bryan).

The majority of determinations, however, came reasonably close to the true value of 20 parts per million. Out of a total of 23 determinations by four analysts, over half (13) were within 10% of the correct value. Fifteen were within 20%, and four showed an error greater than 30%.

WEIGHING ERRORS.

With this method the larger weight of sample taken (100 grams) reduces the error in weighing due to the limitations of the balance to ±0.32 parts per million when a total of 20 parts per million is being estimated. Other weighing errors are correspondingly reduced. An error of 0.0001 gram in weighing the $PbCrO_4$ would produce an error of 0.64 parts per million with the 20 parts per million total.

RECOMMENDATIONS ON REMINGTON MODIFICATION.

From the results obtained with this method, it would appear that the Remington method does not possess quite the accuracy of the Exner method. Consequently it is recommended that no further study be made of the Remington modification method at present.

EXNER METHOD.

VARIATIONS REPORTED.

Still closer checking was found in the results with the Exner method. The greatest variation was from 11 to 22 parts per million with one solution and four analysts, and from 11 to 23 parts per million with one analyst (Bryan), using three solutions. Fifteen out of a total of 21 determinations were within 10% of 20 parts per million, and only three showed an error greater than 20%.

WEIGHING ERRORS.

With the Exner method weighing errors are still further reduced, the error due to weighing limitations of the balance being only ±0.16 parts per million in estimating 20 parts per million of lead. An error of 0.0001 gram in weighing the lead chromate precipitate would give an error of 0.32 parts per million for this 20 parts per million total.

USE OF ALCOHOL IN PRECIPITATING LEAD SULPHATE.

Mr. Pettijohn recommends that alcohol be added in the Exner method, at the point where the $PbSO_4$ (recently formed from the sulphid by the action of nitric and sulphuric acids) is treated with water, in order to precipitate the lead sulphate completely. It appears to the associate referee that this would delay the determination and not add appreciably to the accuracy. The solubility of $PbSO_4$ in water is 0.0042 gram $PbSO_4$ per 100 grams distilled water at 19°C. (Rothmund: Löslichkeit und Löslichkeitsbeeinflussung). This means that when *saturated,* 15 cc. of water would hold only 0.0006 gram of $PbSO_4$. The 15 cc. of water used in transferring the sulphate precipitate does not have time to reach saturation; hence the use of alcohol to recover this minute quantity of $PbSO_4$

is without material effect upon the accuracy of the method in general, if carried out according to Exner's directions. However, where it is desired to use the Exner method with fractional quantities of the 200-gram sample, the error introduced by the loss of the $PbSO_4$ in the transference water might need consideration.

RECOMMENDATIONS ON THE EXNER METHOD.

In view of the shorter time required and the greater accuracy both from a priori considerations and actual experimental data, the Exner method seems well worthy of further study, where a gravimetric method is to be used. In case the full quantity recommended (200 grams) is used, it is advisable to take the substance from the can, thoroughly mix, and secure each complete sample taken for analysis as a composite of many fractions lifted from different portions of the mix.

WICHMANN MODIFICATION OF SEEKER-CLAYTON METHOD.

Mr. H. J. Wichmann in his report presents the following method, which he recommends as dependable:

> One hundred grams of baking powder are weighed out into a 1.3-liter beaker and 10% HCl added to excess in small portions. Excessive frothing may be kept down with ether. The mixture of acid and starch is heated until the starch is hydrolyzed and the solution is quite limpid. After cooling, 200 cc. 50% ammonium citrate solution are added. The solution is kept cold while an excess of ammonia is slowly and carefully added. If a precipitate forms, sufficient ammonium citrate is added to dissolve it. Fifteen cubic centimeters of saturated $HgCl_2$ solution are added and the solution diluted to about 1,200 cc. Hydrogen sulphid is then passed to saturation and the beaker set aside. The precipitate settles rapidly and can readily be filtered. A centrifuge can be used to advantage. There is no need of standing overnight, as the heavy HgS will drag down the PbS. If the solution stands overnight a precipitate of calcium citrate is apt to settle out.
>
> The filter paper with the sulphids is placed in a small casserole, or the sulphids are washed into it from the cylinder, if a centrifuge is used. Ten cubic centimeters of HNO_3 and 2 cc. of concentrated H_2SO_4 are added. The nitric acid is evaporated and the sulphuric acid slowly fumed off. If a gas muffle furnace is available the casserole can be placed on the platform in front of the muffle until the H_2SO_4 has disappeared and then slowly heated inside to a light red heat. The mercury salts will vaporize, and any ferric sulphate present will be broken up to the oxid. After cooling, the lead sulphate is leached out from the residue with an ammoniacal 25% solution of ammonium acetate. Several leachings must be made. The filter paper is then washed with hot water. The filtrate is acidified with acetic acid and the lead precipitated as chromate with potassium dichromate. After standing overnight the lead chromate is collected on a Gooch, dried by heating to 125°C., cooled, and weighed.

In answer to the objection that the mercury would tend to carry off some of the lead with it during volatilization, Mr. Wichmann assures us that he has not found this error to interfere with the accuracy of the method. Mr. Clayton states that in his experience such a volatilization of mercury salts gave low results for the lead; but considering the very much smaller quantities of lead present in each test sample when following the Seeker-Clayton method, it seems likely that the large relative error obtained by Mr. Clayton would become insignificant when using 100-gram samples as recommended by Mr. Wichmann.

RECOMMENDATIONS.

The associate referee recommends that further study be carried out upon the Exner and Wichmann methods. The Exner method is recommended for adoption as a provisional method.

Special emphasis should be laid upon speed of operations, as well as upon clean separations, close measurement of wash water, and accurate weighing. The use of a stirrer during hydrogen sulphid precipitation, followed by centrifuging, should be studied with a view to eliminating the standing overnight. The Wichmann method presents the only gravimetric determination which can be carried out within approximately 24 hours, and is therefore expecially commended for careful study. It is recommended that the determinations be accompanied by a time schedule, showing actual time involved in each operation. This is especially important in view of the large number of samples and the short time available in handling many carloads of material at the factories.

The associate referee wishes to thank the collaborators, T. J. Bryan, J. Hortvet, H. M. Loomis, C. B. Morey, and R. E. Stallings, and the analysts, J. O. Clarke, F. L. Hoyt, E. Pettijohn, and H. J. Wichmann, for their helpful spirit in carrying out the work. Acknowledgment is also made to Mr. G. H. Mains for assistance in discussing data and compiling this report.

THE RÔLE OF CALCIUM SULPHATE IN PHOSPHATE BAKING POWDERS.

(ABSTRACT.)

BY H. E. PATTEN (Bureau of Chemistry, Washington, D. C.).

The present investigation was undertaken in view of the different opinions which have been expressed as to the function of calcium sulphate in phosphate baking powders. Some chemists hold that the calcium sulphate is merely a neutral inert body acting possibly as a drying agent in the baking powder, but contributing nothing to the chemical changes which alone render the powder of service in baking. Others maintain

that the calcium sulphate acts chemically in the dough, and finally is not found in its original form in the finished biscuit, but broken down chemically and recombined into a soluble product (sodium sulphate) and an insoluble product [calcium phosphate $(CaO)_x(P_2O_5)_y$] of variable composition. The presence of calcium sulphate is also held to retard the evolution of gas by the baking powder and thus renders the leavening action more gradual.

There is also question in the minds of some as to whether the reaction between sodium bicarbonate and monocalcium phosphate yields dicalcium phosphate ($CaHPO_4$) or tricalcium phosphate $Ca_3(PO_4)_2$; and also question as to the effect of flour and starch (in the dough of the biscuit) upon the reaction. Indeed some claim that experiments made with sodium bicarbonate, calcium phosphate, calcium sulphate, and water cannot be taken as good evidence of what reactions will take place when these baking-powder ingredients are actually mixed with flour and water and baked into a finished biscuit.

A survey of the literature at the time this work was undertaken[1] showed that accurately controlled heterogeneous equilibria studies had been carried out in which carbonates were investigated as to their action with sulphate and chlorid, but not with phosphate; also that phosphates had been studied in connection with sulphate and chlorid, but not with carbonate. Consequently, there was a large field of reaction possibility still unexplored, even where the baking-powder ingredients alone were taken in aqueous solution, no flour being added.

From these equilibria experiments, and the diagrams graphically representing their data, tentative inferences were made as to the chemical changes which might be expected in the baking reaction, as follows (using symbols for brevity):

If $CaSO_4 \cdot 2H_2O$ or $CaSO_4$ anhydrous be added to $NaHCO_3$ in presence of excess of water—[2]

(1) $CaCO_3$ will be formed (since it is about 1,000 times less soluble than $CaSO_4$) along with Na_2SO_4 and H_2CO_3.

(2) The H_2CO_3 will then decompose and CO_2 gas will be evolved.

(3) The main effect of rise in temperature above 25°C. is to liberate CO_2 held in solution by $CaCO_3$[3], and to deposit the $CaCO_3$ (released by the CO_2) as a precipitate; also temperature rise changes any residual $CaSO_4 \cdot 2H_2O$ to the anhydrous form, $CaSO_4$.

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[1] This problem was referred to the author in 1911, some time in November, by Dr. W. D. Bigelow, at the instance of Dr. H. W. Wiley. A preliminary report was submitted to Dr. Bigelow on January 15, 1912.

[2] Compare U. S. Patent 316,863, April 28, 1885. CO_2 formed from $CaSO_4 +$ alkaline bicarbonates.

[3] $CaH_2(CO_3)_2$ calcium bicarbonate has not been shown to exist, despite the frequent references to it in chemical writings.

If $CaH_4(PO_4)_2$ be added to $NaHCO_3$ in water the reaction varies with the quantity of $NaHCO_3$ used, thus:

(4) $3CaH_4(PO_4)_2 + 4NaHCO_3 = Ca_3(PO_4)_2 + 4NaH_2PO_4 + 4CO_2 + 4H_2O$.
(5) $3CaH_4(PO_4)_2 + 8NaHCO_3 = Ca_3(PO_4)_2 + 4Na_2HPO_4 + 8CO_2 + 8HO$.

It is found that "tricalcium phosphate" is always formed and the variation of $NaHCO_3$ produces a variation in NaH_2PO_4 and Na_2HPO_4 and CO_2 and H_2O.

If we add $CaSO_4$ or $CaSO_4 . 2H_2O$ to $CaH_4(PO_4)_2$ and $NaHCO_3$ in water, the reaction is modified in that an end-product, Na_2HPO_4, is acted upon, thus:

(6) $3CaSO_4 + 3Na_2HPO_4 = Ca_3(PO_4)_2 + 3Na_2SO_4 + H_3PO_4$.

The tricalcium phosphate (a solid solution $(CaO)_x(P_2O_5)_y$ is about 1,000 times less soluble in water than $CaSO_4$, hence this reaction proceeds till the solubility limit of the $Ca_3(PO_4)_2$ in the acid solution resulting is reached. This equation, of course, does not represent the reality, since the composition of the solid solution ($Ca_3(PO_4)_2$?) varies with the composition of the liquid phase in contact with it.

Reaction No. 6 above does not take account of the $NaHCO_3$ present. When this is done we have:

(7) $3CaSO_4 + 2Na_2HPO_4 + 2NaHCO_3 = Ca_3(PO_4)_2 + 3Na_2SO_4 + 2CO_2 + 2H_2O$.

No hint is given in the literature examined of a compound, $CaNa_4(PO_4)_2$, either as a solid phase or in solution.

Without regard to exact equations, such as 4, 5, 6, and 7, it may be inferred from the published isotherms referred to in the literature that—

(8) With calcium sulphate present in excess as a solid phase, very little phosphorus pentoxid will be found in solution.

(9) If the content of $CaSO_4$ is high in the baking powder it is likely that $CaSO_4$ or $CaSO_4 . 2H_2O$ will exist as a solid phase after the baking powder reaction is ended, and some $CaCO_3$ may be present. But if the $CaSO_4$ content is low in the baking powder, then there would be a solid solution left as residue after baking, containing CaO and P_2O_5, but not necessarily in a simple molecular ratio to each other; here, too, some $CaCO_3$ might be left in the solid residue.

(10) Tricalcium phosphate is not permanent or of fixed chemical composition, at least when it is formed in the usual way by precipitation; the P_2O_5 may be washed from it with excess of water, leaving a basic compound, or, conversely, its lime may be extracted by acid solutions, leaving the solid solution relatively higher in P_2O_5 content than in CaO.

(11) The effect of adding salt (sodium chlorid, NaCl) in mixing dough probably is to decrease the P_2O_5 in solution. This effect is observed where chlorids act on calcium phosphates alone in aqueous solution.[1]

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[1] U. S. Dept. Agr., Bur. Soils Bul. 41, p. 34. KCl decomposes $Ca_3(PO_4)_2$ and decreases P_2O_5 in solution.

(12) Precipitation of calcium carbonate in the cold is retarded by the presence of phosphate salts.[1] This would tend, though perhaps only slightly, to hold more CO_2 in solution and give it up at higher temperature in the oven.

The double titration of phosphates, using methyl orange and phenolphthalein and a neutral salt such as calcium chlorid, is outlined in detail by H. N. Morse in his Analytical Chemistry, and has long been a matter of common knowledge. Wadman[2] in 1894 published results on testing phosphate baking powders, using essentially this method.

Although the foregoing review of the subject makes it appear that calcium sulphate is not inert in the baking-powder reaction, a few experiments have been carried out to ascertain the effect of the calcium sulphate, not only in quantitative experiments with the chemicals and water but also in baking tests.

The following table shows the amount of calcium sulphate not decomposed when varying amounts were used in the baking-powder reaction in flasks with given amounts of monocalcium phosphate and sodium bicarbonate.

Quantity of calcium sulphate left in solid residue after baking-powder reaction.[1]

CALCIUM SULPHATE ADDED		CALCIUM SULPHATE LEFT IN SOLID RESIDUE
Grams	Per cent by weight of $CaH_4(PO_4)_2.H_2O$	Gram
0.378	15	0
0.756	30	0.054
1.009	40	0.080
1.512	60	0.175
2.018	80	0.545

[1] Reaction: 2.5215 grams $3CaH_4(PO_4)_2.H_2O$ + 2.2460 grams 8 $NaHCO_3$ + (Q.) $CaSO_4$ + 100 grams H_2O.

It appears that with the proportions of monocalcium phosphate and sodium bicarbonate used, the calcium sulphate was entirely decomposed when its amount as $CaSO_4$ was 15% of the weight of the phosphate ($CaH_4(PO_4)_2.H_2O$).

In regard to the effect of $CaSO_4$ upon the production of carbon dioxid, it may be said that since both calcium sulphate and monocalcium phosphate react with sodium bicarbonate, giving off carbon dioxid, when mixed they will still react, as shown in equations 4, 5, 6, and 7 above, which in general are borne out in our experiments, results subject, of course, to variations produced by change in concentrations and temperatures.

Further, a monocalcium phosphate which has been neutralized to phenolphthalein by addition of N/10 sodium hydroxid shows a marked increase in acidity when calcium sulphate is added. This excess acidity may be titrated with standard alkali, or it may be determined quantitatively by the electrometric method, using the hydrogen electrode to evaluate the increase in the so-called "hydrogen ion concentration." Both methods were used in this investigation.

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[1] Storer, Am. J. Sci., 1858, **35**: (2) 41; Dulong, Ann. Chim., 1812, **82**: 273; Spiller, J. Chem. Soc., 1858, **10**: 110; Kippenberger, Z. anorg. Chem., 1894, **6**: 177.

[2] J. Am. Chem. Soc., 1894, **16**: 333.

The electrometric method has the advantage that it does not disturb the equilibrium as does the addition of alkali in titrating.

Baking experiments conducted in coöperation with Miss Hannah Wessling confirmed the conclusions from the chemical studies. Addition of calcium sulphate appreciably raised the limit of alkali which could be used with a given amount of phosphate in the baking-powder mixture without leaving an alkaline taste in the biscuit.

The experiments reported at this time have settled only the question as to whether the calcium sulphate does take part in the reaction. The exact limits of the reaction are yet to be determined.

REPORT ON MEAT AND FISH.

By E. D. Clark (Food Research Laboratory, Philadelphia, Pa.),
Associate Referee.

Studies this year are included in two groups. The first was to determine the accuracy and general application of the Price method for starch in meat products by the analysis of known samples through the aid of the collaborators. The second group of studies included methods of detecting incipient decomposition and changes in fish flesh by determining the various forms of nitrogen in the so-called "nitrogen distribution," the changes in fat constants, etc.

PRICE METHOD FOR STARCH IN MEAT PRODUCTS.

For the last two years it has been recommended by the association that Price's method be studied further, with a view to its adoption as provisional. It seemed desirable to make a final study of this method before recommending its adoption. Previous work reported on the method shows that the directions as given are easily carried out and that theoretical values are obtained for known weights of starch added to meat. Accordingly, the immediate problem seemed to be a study of the uniformity of results obtained on the same meat sample by different analysts, rather than any further investigation of theoretical considerations.

In collaborative work of this sort on starch in meat it is impossible to prepare uniform and exact samples because of the difficulty of thoroughly mixing dry starch or cereal with a wet and greasy material like sausage meat. Accordingly, two large samples of ground meat were prepared, using borax as a preservative. To each of these samples potato starch was added and the whole thoroughly mixed by running through a meat chopper ten times in succession. Allowing for the moisture, etc., in the starch, it was possible to prepare fairly uniform samples of chopped meat containing an approximately known weight of starch. Portions of the large samples were analyzed for starch, and then 4-ounce samples were sent out to collaborators.

In the original Price method, reduced copper is determined by titration, as proposed by Low. However, in many laboratories it is customary to determine the precipitated cuprous oxid by weighing directly, according to Munson and Walker's procedure. Therefore, in order to get comparative figures, the collaborators were requested to determine the starch in the second sample by the use of both the volumetric and gravimetric methods for reduced copper.

Results of collaborative work on the Price method for starch in meat products.

ANALYST	AVERAGES OF TWO OR MORE DETERMINATIONS			
	Sample 1 (± 1.30% starch)		Sample 2 (± 1.65% starch)	
	Volumetric	Gravimetric	Volumetric	Gravimetric
	per cent	*per cent*	*per cent*	*per cent*
L. H. Almy, Philadelphia	1.25	1.23	1.67	1.69
E. D. Clark, Philadelphia	1.22	....	1.42	1.45
R. D. Cook, Chicago	1.24	....	1.44	....
M. O. Johnson, Washington	1.33	....	....	....
T. R. Le Compte, Washington	....	....	1.67	1.65
R. M. Mehurin, Washington	....	....	1.57	1.53
C. G. Sutton, St. Paul	1.11	1.11	1.42	1.47
A. S. Thatcher, Washington	1.36	....	....	....
G. W. Trainor, Chicago	1.28	....	1.74	....
Average	1.26	1.17	1.56	1.56

A study of the collaborators' figures shows that they are in satisfactory agreement with each other and with the approximately known weight of starch added in making up the sample. The Price method has an accuracy and an ease of manipulation that make it worthy of adoption as a provisional method. To accommodate those analysts who prefer to determine the copper gravimetrically as cuprous oxid, rather than by titrating according to Low's method, the Price method should be modified to permit the weighing of cuprous oxid as an optional procedure. From our experience, and that of our collaborators, it seems that this method should be adopted as provisional in place of that of Mayrhofer.

PRELIMINARY STUDIES ON CHEMICAL METHODS OF DETECTING DETERIORATION IN FISH FLESH.

By E. D. Clark and L. H. Almy.

The quantitative study of changes in fish flesh upon standing or during cold storage is a comparatively new one. The researches of Richardson and Scherubel, Emmett and Grindley, and others, upon meat during storage opened the way in that field. Wiley, Pennington, and collaborators, have done the fundamental work on the chemistry of decomposition processes in the flesh of poultry, game, etc. The investigations of Smith[1] and of Perlzweig and Gies[2] on changes in fish during cold storage are the pioneer contributions to our knowledge of the behavior of fish flesh under refrigeration.

Owing to the fact that there were few generally accepted methods for studying chemical changes in fish flesh, and also that the perishable nature of fresh fish made it impossible to send samples to collaborators, it was decided to make the preliminary studies in our own laboratory. This work was done in connection with the investigations of the laboratory on the effects of cold storage upon fish. Therefore, this report is a result of the year's experience with various methods applied to the study of fish.

The following list shows the determinations made for the tabulation of results on changes in the fish during cold storage. It was intended to include every determination that offered any possibility of giving information about fish flesh and its changes. Some of these determinations are unnecessary and some may be of little value, but all of them will help to give definite information about the adequacy of the methods themselves as applied to this problem. Most of the determinations have been adopted from the work of early investigators upon meats and poultry. The determination of the acidity of the fish extract was suggested by Smith's publication. Determination of amino acids by both the Van Slyke and Sörenson methods was a new departure. Other new methods or modifications will be apparent.

Tabulation of complete analysis of fish.

(A) SAMPLE.

1. Description of fish and history.
2. Total weight.
3. Edible portion.
4. Refuse.

(B) GROSS ANALYSIS.

1. Moisture.
2. Solids.
3. Ash.
4. Ether extract.
5. Total nitrogen
6. Nitrogen × 6.25 (protein).
7. Water-insoluble nitrogen.
8. Ammonia and amine nitrogen by aëration.

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[1] Smith. Biochem. Bul., 1913, **3**: 54.

[2] Perlzweig and Gies. Biochem. Bul., 1913, **3**: 69.

(C) ANALYSIS OF AQUEOUS EXTRACT.

1. Acidity of extract in terms of N/10 NaOH solution with phenolphthalein.
2. Solids.
3. Ash.
4. Organic matter.
5. Nitrogen distribution in extract.
    - (a) Total nitrogen of extract, or soluble nitrogen.
    - (b) Coagulable nitrogen.
    - (c) Nitrogen precipitated by—
        - (I) Tannin-salt method.
        - (II) Zinc sulphate method.
    - (d) Nitrogen "bases," etc., by difference.
    - (e) "Amino-acid nitrogen" by—
        - (I) Van Slyke method.
        - (II) Sörensen titration method.
    - (f) Ammonia and amine nitrogen by aëration.

(D) STUDY OF FAT EXTRACTED FROM FLESH.

1. Specific gravity.
2. Index of refraction.
3. Iodin number.
4. Saponification number.
5. Acid number.
6. Acetyl number.

There is no need to discuss the ordinary determinations like total nitrogen, ash, fat constants, etc., but in the case of ammonia nitrogen there are certain complications that must be considered. In the determination of the so-called ammonia nitrogen we used a modification of the Folin aëration method. We followed Smith in using the Steel[1] modification of Folin's method because Perlzweig and Gies reported the presence of crystals of ammonium magnesium phosphate in fish flesh even early in the process of deterioration. The use of sodium hydroxid and sodium chlorid in place of the sodium carbonate, as suggested by Steel, seems to be necessary in cases like the analysis of fish where magnesium ammonium phosphate is known to be present. Benedict and Osterberg[2] have made a detailed study of the use of Steel's modification as well as the original Folin method upon urines, and they report that the former method apparently gives correct results for ammonia under nearly every condition. In fish flesh not only do we have the interference of this magnesium compound but also the added complication of amines, unknown as to nature and amount. Later we will show that certain classes of amines may be determined quantitatively by the aëration method, either when alone or with ammonium salts. Since this is true, and since we know that amines occur in fish, we found it desirable to use Steel's modification, and later to make a study of its efficiency in determining amines, either in the presence or absence of ammonium salts.

In order to study the interference, if any, of the amines with the determination of ammonia, we carried out the following experiments: Standard solutions of methyl amine, trimethyl amine, and ammonium chlorid were prepared. The exact amount of nitrogen contained in 5 cc. portions of these solutions was ascertained by duplicate Kjeldahl determinations. Then the same amounts of these standard solutions were analyzed by the Folin aëration method, using Steel's modification, as already noted. These solutions were analyzed separately and mixed in stated proportions. The figures by the aëration method are the averages of four separate determinations.

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[1] Steel. J. Biol. Chem., 1910, **8**: 365.

[2] Benedict and Osterberg. Biochem. Bul., 1913, **3**: 47.

Determination of ammonia and amine nitrogen by the aëration method.

SUBSTANCE USED	NITROGEN BY AËRATION	NITROGEN BY KJELDAHL METHOD
	gram	*gram*
Methyl amine solution alone	0.0052	0.0050
Trimethyl amine solution alone	0.0072	0.0077
Ammonium chlorid solution alone	0.0189	0.0188
Trimethyl amine + NH_4Cl, solutions mixed	0.0148	0.0143
Trimethyl amine + CH_3NH_2, solutions mixed	0.0124	0.0127

It is evident from these results that the primary or tertiary amines, either alone or with ammonium salts, are determined quantitatively by the modified Folin method for ammonium nitrogen. Therefore, we consider that determinations by this method give "ammonia and amine" nitrogen. Proteins do not interfere.

We already have seen that the presence of amines and magnesium compounds in fish is a disturbing factor in the determination of the so-called "ammonia" nitrogen by the aëration method. In the past it usually has been customary to study the amino-acid content of flesh extracts when inquiring into the effect of cold storage upon such material. In our own work we used the Sörensen[1] titration method for amino acids in making parallel determinations along with the gasometric method of Van Slyke.[2] In determining amino acids in fish flesh, account must be taken of the presence of both ammonia and amines, which are very closely related to the amino acids, and have many properties in common with them. For this reason a study was made of the effect of ammonia and amines, separately and in combination, upon the Sörensen titration.

Our study of the behavior of ammonia and amines in amino-acid titrations was carried out in much the same way as that upon the aëration method for ammonia described above. Standard solutions of leucin, of ammonia, and of different amines were prepared and analyzed for their "amino-acid nitrogen" according to the Sörensen method. Later, definite volumes of these same solutions were mixed and then analyzed for amino-acid nitrogen in the same way. It is obvious that if there were no interference between the ammonia, amines, and amino acids the results of the titrations upon the mixed solutions would be the same as those upon

[1] Sörensen. Biochem. Z., 1907–8, **7**: 44.

[2] Van Slyke. J. Biol. Chem., 1912, **12**: 275, and idem., 1913, **16**: 121.

the solutions analyzed separately. Following are some results of this work:

Effect of ammonium chlorid and amines upon the determination of amino-acid nitrogen by the Sörensen method.

SUBSTANCES USED	NITROGEN DETERMINED SEPARATELY	NITROGEN DETERMINED IN MIXTURE
	gram	*gram*
Leucin solution alone	0.0059	....
Methyl amine solution alone	0.0072	....
Trimethyl amine solution alone	0.0017	....
Ammonium chlorid solution alone	0.0089	....
Leucin + NH_4Cl, solutions mixed	0.0148	0.0148
Leucin + CH_3NH_2, solutions mixed	0.0131	0.0133
Leucin + $(CH_3)_3N$, solutions mixed	0.0076	0.0076
Leucin + CH_3NH_2 + NH_4Cl, solutions mixed	0.0219	0.0215
Leucin + $(CH_3)_3N$ + NH_4Cl, solutions mixed	0.0165	0.0159

An inspection of this table shows that the presence of ammonia in relatively small quantities does not seriously interfere with the amino-acid determination; neither does the presence of amines; but when amines and ammonia occur together the results obtained by the Sörensen titration are not reliable. Unfortunately, this is exactly the state of affairs existing in fish flesh. This work upon the interference of certain nitrogen compounds with the Sörensen amino-acid determination is a confirmation of the observations made by Henriques and Sörensen[1] upon the disturbing influence of ammonium salts and methyl amine. It is evident, then, that the determination of amino acids by the above method gives results that are inaccurate owing to the interference on the part of ammonia and amines. This interference, unfortunately, is not a definite one, and for that reason it is not allowable to correct the amino-acid results by subtracting the amount of nitrogen present as ammonia plus amines, determined by the aëration method. Therefore, in all the tables the percentage of amino acid as reported is subject to a varying and unknown correction, due to the presence of ammonia and amines in the fish flesh.

It is well known to organic chemists that various types of amines react in different and characteristic ways with nitrous acid; therefore, the Van Slyke gasometric method for amino acids is likewise subject to correction on account of the presence of various types of amines in fish flesh. If the time allowed for the reaction between the nitrous acid and the substance being analyzed is sufficiently long we know that the ammonia will be determined quantitatively by the Van Slyke method. However, it is not customary to allow the reaction to run more than three or five minutes. For that reason our results for amino acids determined by the Van Slyke method are also subject to correction by an unknown amount, due to the presence in fish flesh of different types of amines and ammonia, and must not be viewed as giving accurate information on the percentage of amino acids alone.

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[1] Henriques and Sörensen. Z. physiol. Chem., 1910, **64**: 120.

To make the Sörensen and Van Slyke determinations of amino acids entirely accurate, it would, of course, have been possible to remove the ammonia and amines by distilling with calcium hydroxid according to Van Slyke's procedure. But this precaution seems hardly necessary in our work where the quantities of amino acids and ammonia are about 0.15% and 0.01%, respectively. Furthermore, the comparative value of our uncorrected amino-acid figures on extracts of fish flesh in studies of nitrogen distribution are not impaired, because the determinations are always made in exactly the same way on nearly identical material at definite periods during two years.

Many parallel determinations were made of nitrogen precipitated by the tannin-salt reagent and by zinc sulphate applied to the fish extracts. The results obtained by the latter method were constantly lower than those from the tannin-salt method. It is known that the zinc sulphate precipitates most sharply those substances commonly classed as proteoses, while the other method includes certain other nitrogenous substances as well as proteoses. However, in our work we always use both methods in order to have comparative figures running through the whole period of cold storage. We have found it very convenient and accurate to filter off the zinc sulphate precipitate on an asbestos mat in a Gooch crucible, wash mat and precipitate into a Kjeldahl flask and transfer for the nitrogen determinations.

In the space at our disposal it is impossible to indicate the details of analytical procedures used in our various determinations, as listed on pages 231–232. The strict precautions to be followed in preparing aqueous extracts of fish flesh, preserving them, taking aliquots, etc., may also be omitted at this time. This is also true of the details of extracting and analyzing the oils from the fish. In another year the results of all our work on frozen fish stored for two years will be completed and tabulated. At that time the comparative value of all the different determinations in showing changes in fish flesh will become evident and may be discussed more intelligently.

It is obvious that all of the methods so far applied in our work on fish aim to detect deterioration by changes in constituents *already present*, caused either by the action of bacteria or enzymes in the fish itself. For many purposes it seems very desirable to find methods that will show deterioration by the detection of decomposition products *not found* in perfectly fresh fish. We have in mind such things as indol, skatol, phenolic substances, and similar products of protein decomposition. The evidence of the early stages of decomposition in fish is not easy to get, and no stone should be left unturned in a search for indications of the tainted condition in this class of foods.

RECOMMENDATIONS.

It is recommended—

(1) That Price's method (U. S. Bur. Chem. Circ. 108, p. 10) be made a provisional method for starch in meat products in place of Mayrhofer's method (U. S. Bur. Chem. Bul. 107 (rev.), p. 109, (b) (2)). With very little modification from Price's description, the official description should be:

In a 200 cc. beaker treat 10 grams of finely divided meat with 75 cc. of an 8% solution of potassium hydrate in 95% alcohol, and heat on the steam bath until all the meat is dissolved. This will require from 30 to 45 minutes. Add an equal volume of 95% alcohol, cool, and allow to stand at least one hour. Filter by suction through a thin layer of asbestos in a Gooch crucible. Wash twice with warm 4% potassium hydrate in 50% alcohol and then twice with warm 50% alcohol. Discard the washings. Endeavor to retain as much of the precipitate in the beaker as possible until the last washing. Place the crucible with contents in the original beaker, add 40 cc. of water and then 25 cc. of concentrated sulphuric acid. Stir during the addition of the acid and see that the acid comes in contact with all the precipitate. Allow to stand about 5 minutes, add 40 cc. of water, and heat just to boiling, stirring constantly. Transfer the solution to a 500 cc. graduated flask, add 2 cc. of a 20% aqueous solution of phosphotungstic acid, allow to cool to room temperature, and make up to mark with distilled water. Filter through a starch-free filter paper, and determine the dextrose present in a 50 cc. portion of the filtrate with Fehling's solution after neutralizing the acid, using Low's method, as given in U. S. Bureau of Chemistry Bulletin 107 (revised), page 241, for the determination of the copper in the cuprous oxid precipitate, or the latter may be weighed as directed on page 242. The amount of dextrose multiplied by 0.9 gives the equivalent in starch.

(2) That comparative studies be continued of the different determinations of the nitrogen distribution and the best manner of carrying them out as applied to extracts of fish flesh. Efforts should be made to standardize the method of determining coagulable proteins in such work. New methods proposed for the determination of proteoses, amino acids, amines, etc., in biological material should be investigated with a view to finding more sensitive and more accurate indices of deterioration in fish.

(3) That a study be made of the value of volatile distillation products like amines, indol, etc., in detecting incipient decomposition in fish.

REPORT ON FATS AND OILS.

By R. H. Kerr (Bureau of Animal Industry, Washington, D. C.), *Associate Referee.*

The attention of the associate referee on fats and oils has been given to a careful consideration of some of the new problems which have been raised by the rapid increase in the commercial practice of hardening oils by hydrogen. While the practice so far has been applied mainly to cottonseed oil, almost any edible oil may have its value so enhanced by this treatment as to make its hydrogenation a source of profit. It must be assumed, then, that the problems of those unlucky chemists who are responsible for detections of the adulteration of edible oils and fats are now to be increased and complicated by the addition to the list of possible adulterants of a long list of hydrogenated and partly hydrogenated fats. Aside from the evident fact that the would-be adulterator may now alter at will the iodin number, refractive index, and titer of any fat he may choose, the process of hydrogenation brings about other changes which are of far-reaching importance. The Halphen test of cottonseed oil disappears, the erucic acid of rape and mustard oils becomes bebenic acid, the highly unsaturated acids of the fish oils are reduced to solid acids which no longer give the characteristic precipitate of insoluble bromids. It is to be seen at once that many of our tried and trusty tests will fail us completely before this new problem, and it is equally plain that we must devise new tests to meet the new condition or confess failure.

One of the most striking features of the problem is the absolute lack of definite information regarding the composition of fats. Although fats have been studied and worked over by chemists since the time of Chevreul, and the literature of the subject comprises a large number of bulky volumes, it remains true today that we do not know accurately the composition of a single fat. We know that cottonseed oil, for example, consists of the glycerids of palmitic, oleic, and linolic acids, but we do not know whether it consists of a simple mixture of the three triglycerids of these acids, or whether the whole array of possible mixed glycerids may not also be present. In fact, I am not aware that one single glycerid ever has been isolated from cottonseed oil and identified. Almost as much may be said of many other well-known fats. It was not known until two years ago that lard did not contain the glycerid tristearin despite all the thousands of analyses of lard made every year.

In dealing with the problem raised by the use of hydrogenated fats, it appears to me that the first requirement will be a much more extensive and intimate knowledge of the exact composition of fats. When we know of what glycerids a fat is composed we are then in a position to recognize that fat wherever we find it, tedious and difficult as the process of identification may be. Accurate knowledge may, however, point the way to short and simple tests, just as Boemer's tedious fractional crystallization of lard finally led him to a method only a little less simple and speedy than the method already adopted by this association for the detection of the adulteration of lard. A modification of this method which I hope to present to this association at a later meeting will set the detection of beef fat, mutton fat, and all of the hydrogenated oils in lard upon a firm foundation and also will enable us to distinguish between natural lard and lard hardened by addition of hydrogen. The method, if presented, will be offered as a method for the detection of the glycerid tristearin in lard, not as a method for the detection of beef fat.

Our needs appear, then, to be (1) the collection of a large amount of wholly new information, and (2) new and less laborious methods whereby the needful knowledge may be acquired. It is along the second line that I have been working. The work I have been doing has not yet reached the stage of definite methods of analysis available for coöperative study, but some progress has been made along that line, and some improved methods probably will be available for study during the coming year. The ones suggested at present are:

(1) A better method for the detection and identification of tristearin.

(2) A better method for the separation of solid and liquid fatty acids.

(3) A method for the separation of the liquid fatty acids from each other.

(4) A method for the detection of cholesterol in mixture of animal and vegetable fats.

It is hoped that at least one, and possibly two, of these methods may be studied coöperatively and the results of the study presented at the next meeting of the association.

Mr. Hand at this point explained the operation of an apparatus for the extraction of fats which he exhibited.

REPORT ON DAIRY PRODUCTS (ADULTERATION).

By Julius Hortvet (State Dairy and Food Department, St. Paul, Minn.), *Associate Referee.*

The work of the past two years has magnified the importance of a special study of methods for the determination of fat in dairy products. It has, in fact, been realized more than heretofore that we are in urgent need of a thorough investigation of methods which have been recommended for study by this association; and, having in view the importance of advancing our work from year to year along the lines indicated by these recommendations, attention is first directed to the committee reports on this subject since 1906:

(1) At the meeting held in November, 1906, it was recommended, "That the Gottlieb method (Landw. Vers.-Sta., 1892, **40**: 1) be made provisional for the determination of fat in milk. Referred to referee for 1907 for further recommendation. Adopted."[1]

(2) At the meeting held in October, 1907, it was recommended that the study of methods of analysis of condensed milk be continued and, among other things, special attention be given to the "Roese-Gottlieb method for the determination of fat as described in the proceedings of the association for 1906. Adopted."[2]

(3) At the meeting held in November, 1908, it was recommended, "That the determination of fat in condensed milk be studied, special attention being given to solutions of less than 20% concentration."[3]

(4) At the meeting held in August, 1909, it was recommended, "That methods for determining fat in both sweetened and unsweetened condensed milk be studied, giving special attention to those modifications of the Babcock and extraction methods recently brought out in Bulletin 134 of the Indiana Agricultural Experiment Station. Adopted."[4]

(5) At the meeting held in November, 1910, it was recommended, "(1) That the Roese-Gottlieb method be adopted as provisional for the determination of fat in milks and condensed milk, both sweetened and unsweetened. Carried. (Recommended to the committee by G. E. Patrick as official.) (2) That the Roese-Gottlieb method be further studied for the analysis of ice cream, milk powders, malted milks, and milk chocolates. Adopted."[5]

(6) At the meeting held in November, 1911, it was recommended, "That Paul's method of extracting fat from dairy products be further studied, and that the fat obtained by this method be studied as to its chemical and physical constants. Adopted."[6]

During the meetings held in 1912, 1913, and 1914, the reports of the associate referee on dairy products were devoted chiefly to a continuation of the study of Paul's continuous-extraction method, together with the modifications of the method which have been proposed. Examination of the work on dairy products during the past eight years reveals the fact that the recommendations adopted during that period have not been consistently carried out. In fact, so far as the proceedings of the past eight years indicate, the Roese-Gottlieb method has been adopted only as provisional. It appears to have been recommended for adoption as official, but, so far as can be learned, no further definite action along that line has been taken. Also, the further recommendations for study of the Roese-Gottlieb method and other methods for the determination of fat in ice cream, milk powders, malted milk, etc., appear to have been overlooked. It therefore has been the aim of the associate referee during the past season to take up the study of methods for determining fat in dairy products along the lines recommended by this association between the years 1906 and 1911. The work on Paul's continuous-extraction method was practically disposed of at the meeting held in November, 1914, and no definite recommendation for the final adoption of that method was made, although it appeared that the method was valuable and serviceable in certain cases and for the special purpose for which it was originally designed. Taking up the work on dairy products at the stage indicated in the proceedings of 1911, the associate referee has accordingly enlisted the assistance of about twenty collaborators in a further study of the Roese-Gottlieb method, with a view to placing this method in line for final adoption as official in 1916. Also, following out the instructions of this association as implied in the recommendations adopted at the meetings held in 1909 and 1910, the plan of work of the past season has included a comparative study of several modifications of the Babcock method which have been proposed during recent years. The following instructions were sent out to the collaborators:

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[1] U. S. Bur. Chem. Bul. 105, p. 154.

[2] Ibid., 116, p. 117.

[3] Ibid., 122, p. 188.

[4] Ibid., 132, p. 188.

[5] Ibid., 137, p. 169.

[6] Ibid., 152, p. 188.

INSTRUCTIONS TO COLLABORATORS.

Herewith I am sending you directions for collaborative work on dairy products for 1915. These directions include a description of various methods which have been proposed for the determination of fat in evaporated milk and ice cream. Also there is included a description of the Roese-Gottlieb method, together with general instructions. You are requested to make a comparative study of all of these methods, and for that purpose to obtain in the local market two samples of each of the products named in the directions, viz., *whole milk, sweet cream, evaporated milk,* and *ice cream.* Instead of attempting to send out uniform samples to the collaborators, the above plan has been decided upon as preferable. Each collaborator will then carry out his work on samples obtained by himself, and, in addition, if time permits, may extend the work on as many additional samples as he may wish to investigate. You are requested to report your results in the plan shown in the tabular form herewith inclosed, and to write out in full your discussions and criticisms on the various methods.

During recent years a number of methods have been devised for testing butterfat in dairy products, and a considerable amount of uncertainty has arisen regarding the reliability of standard methods heretofore employed. It therefore has been deemed advisable to subject several of the most important of these tests to a careful critical study alongside of the well-tried-out Roese-Gottlieb procedure adopted as provisional a few years ago. There is an urgent call for a reliable centrifugal method for condensed milk and ice cream, and it is hoped that the collaborative work of the present season will bring about some agreement, at least on essential points, in connection with one or more of the methods which have been proposed.

Later in the season, as a result of correspondence with Dr. W. D. Bigelow, chief chemist of the National Canners' Association Laboratory, it was decided to supplement the work outlined in these instructions by determinations to be carried out on specially prepared samples of evaporated and condensed milk. These samples were put up under the careful supervision of Dr. Bigelow, at the factory of Borden's Condensed Milk Company, at Norwich, N. Y., and sent out direct to the collaborators. The samples included: (1) An unsweetened evaporated milk, (2) a sweetened condensed milk, (3) a sweetened condensed milk of composition different from sample 2. In connection with these samples, the following additional instructions were sent to the collaborators:

> In addition to the work outlined in the instructions which I sent you some time ago, I will request that you make determinations of butterfat on these three samples by the Roese-Gottlieb method, and, in addition, by any other method which you believe may give correct results. You may select any one of the methods described in the outline sent you; or if you have another method which you have employed in your laboratory, I should be pleased to have you report results by that method. Results obtained on these samples are to be reported at the same time that you send me your statement covering the work included in my outline already sent you.

Carrying out the plan of work as originally outlined in the first letter of instructions, the collaborators were given the following description of methods:

DESCRIPTION OF METHODS.

BRINSMAID METHOD.

Place 2 cc. of glycerin in a Babcock milk test bottle and rotate so that the lower half surface is coated with the glycerin. In a small beaker mix 12 cc. of sulphuric acid (C. P., sp. gr. 1.84) with 18 cc. of 80% acetic acid. Weigh 9 grams of the well-mixed sample into the bottle and pour in the mixed acids so that the neck of the bottle is well washed down, then rotate the bottle so that the milk and acids are mixed and all white lumps dissolved. After thorough mixing, set the bottle aside for 15 minutes, the mixture in the meantime becoming black as in the ordinary Babcock test. Arrange a suitable rack or support so that the bottle may be placed standing in boiling water, allowing water of sufficient depth to cover the bottle above its contents. Leave the bottle in the water for 20 minutes, mixing the contents several times during this period, run in the centrifuge 5 minutes, add hot water so that the contents of the bottle rise to the bottom of the neck, then run 2 minutes, add hot water until the fat rises to the upper part of the neck, and complete the test by making a final run of 1 minute. Measure the fat column and express the result in the manner described under the heading, "Reading the fat column."

Notes on the method.—The preliminary coating of the inside of the bottle with glycerin to some extent prevents the milk from adhering to the sides. A good method of introducing the glycerin into the bottle is to take an ordinary 2 cc. pipette, cut off the tapering point, draw the glycerin into the pipette, wipe off the outside around the end, place the pipette down into the bottle, and allow the glycerin to drain out. This procedure will prevent the glycerin from coming in contact with the neck of the bottle. Considerable heat is developed when the acids are mixed, but there is no danger of the mixture becoming too hot. A thorough mixing of the milk and acids is necessary for good results. This method appears to have been in use for several years in the laboratory of the Illinois Dairy and Food Commission. Mr. David Klein, Illinois State analyst, proposes that in the case of condensed milk a correction figure of 0.24 be subtracted from the result after multiplying the reading by 2.

GRIGSBY METHOD.

Weigh 9 grams of the thoroughly mixed sample into a Babcock milk test bottle and add 10 cc. of glacial acetic acid, washing down the neck of the bottle with the acid, then heat the mixture by putting the body of the bottle in hot water until the contents appear smooth, uniform, and free from lumps. While hot, add ordinary Babcock sulphuric acid, about 1 or 2 cc. at a time, mixing the contents after each addition, until the color of the mixture becomes dark chocolate brown, centrifuge 5 minutes, run in hot water to the bottom of the neck, centrifuge 2 minutes longer, then run in hot water until the fat column comes nearly to the top of the neck, and complete with a final run in the centrifuge of 1 minute. Measure the fat column and express the result as directed in the paragraph headed, "Reading the fat column."

Notes on the method.—Too long heating or overheating of the mixture of acetic acid and sample has practically no effect on the result, heating for 5 to 10 minutes in water slightly below boiling temperature being usually sufficient. The hotter the mixture the less sulphuric acid will ordinarily be required to produce the proper result. The fat column should be perfectly clear and free from curd or char. In case either curd or char appears, the test should be repeated, adding sulphuric acid so as to produce a darker color in the case of interfering curd or so as to produce a lighter color in the case of interfering char.

WENDLER METHOD[1].

Prepare a reagent having the following composition: 125 grams sodium hydroxid, 25 grams Rochelle salt, 25 grams sodium chlorid; dissolve in water, mix the solution, and make volume up to 500 cc. In testing whole milk, run 19 cc. of the reagent into a Babcock test bottle, add 17.6 cc. of the sample in such manner as to float over the reagent, then add 10 or 12 drops of isobutyl alcohol. In testing cream, weigh 18 grams of the sample into the test bottle, run in 19 cc. of the reagent so as to displace the cream, then add 10 or 12 drops of isobutyl alcohol. In testing evaporated milk, weigh 9 grams of the sample in the bottle, add 9 cc. of water and thoroughly mix, then run in 19 cc. of reagent so as to displace the sample, and add 10 or 12 drops of isobutyl alcohol. In testing ice cream proceed as in the case of evaporated milk. In each case, after introducing sample and reagents, shake the mixture for several minutes, place the test bottle in a water bath at 50°C. for 3 to 5 minutes in the case of milk and cream, and in the case of evaporated milk and ice cream heat the mixture at 60°C. 5 to 7 minutes. Shake thoroughly during the heating, then centrifuge at highest possible speed for 4 minutes. Add boiling hot water to bring the fat column up to the graduations in the neck, centrifuge an additional minute, measure the fat column, and express results as directed in paragraph headed, "Reading the fat column."

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[1] Z. öffent. Chem., 1906, **12**: 41–58.

In addition to the methods to be subjected to comparative study described above, determine fat in each sample according to the Roese-Gottlieb method. Also, in the case of milk and cream samples make the fat determination according to the Babcock method carried out according to procedure commonly recognized as correct.

For convenience in making comparative tests by the Roese-Gottlieb method, the following description is added.

ROESE-GOTTLIEB METHOD.

Weigh 40 grams of the properly prepared sample, preferably in a tared weighing dish used for sugar analysis, transfer by washing to a 100 cc. graduated sugar flask, and make up to the mark with water. Measure 10 cc. of this solution into a Röhrig tube or into a suitable size Werner-Schmidt extraction apparatus, using for the purpose not more than 10 cc. of water to transfer sample to the tube. To the material in the extraction tube add 1.25 cc. of concentrated ammonium hydroxid (2 cc. if the sample be sour) and mix thoroughly. Add 10 cc. of 95% alcohol and mix well, then add 25 cc. of washed ethyl ether, shake vigorously for half a minute, add 25 cc. of petroleum ether (redistilled slowly at a temperature below 60° preferably) and shake for half a minute. Let stand 20 minutes, or until the upper liquid is practically clear and its lower level constant. Draw off the ether-fat solution as much as possible (usually 0.5 to 0.9 will be left) into a weighed flask through a small quick-acting filter. Re-extract the liquid remaining in the tube, this time with only 15 cc. of each ether, shaking vigorously half a minute, and allow to settle. Draw off the clear solution through the small filter into the same flask as before and wash the tip of the outlet, the funnel, and filter with a few cc. of a mixture of the two ethers in equal parts. Extract again and wash in the manner just described. Evaporate the ether slowly on a steam bath, then dry the fat in a water-oven until loss of weight ceases.

Notes on the method.—Cream and evaporated milk are best sampled by taking 40 grams and dilute to 100 cc. A 10 cc. sample of this mixture is taken for analysis. In the case of ice cream or other products which are not smooth and which contain air bubbles, 4 grams of the thoroughly mixed sample are weighed and introduced into a Röhrig tube with the aid of 10 cc. of water, then ammonia, alcohol, and ether are added in the amounts called for by the method. In the case of whole milk it has been found convenient to weigh out 10 grams directly. If an emulsion occurs in the Röhrig tube the addition of a little alcohol followed by shaking will usually break it up. Also, the use of a tube of larger caliber will tend to overcome this difficulty. Or, use a large test tube provided with a blowing-off device, as in the Werner-Schmidt method. Care should be taken to weigh the residue after evaporation of ether under exactly the same conditions as those under which the dish was weighed. If the fat contains casein or foreign matter, re-extract with ether, evaporate, and weigh again. If all proper precautions are taken, results on a given sample should check within 0.02 to 0.05%. If the result is not satisfactory repeat the analysis.

GENERAL INSTRUCTIONS.

(1) *Condition of samples.*—All samples should be in the best condition possible. An evaporated milk should be perfectly smooth and contain no small lumps of fat or curded matter.

(2) *Sampling ice cream.*—Allow the sample to soften at room temperature, with frequent stirring with a spoon. A good method of obtaining a uniform sample is

to mix with an egg beater just before weighing. It has been found difficult to obtain a fair sample after the ice cream has been heated on the water bath, owing to the fact that the butterfat is melted and quickly rises to the surface.

(3) *Babcock test bottle.*—Use an 18-gram milk test bottle, reading to 8% and graduated to 0.1 of 1%.

(4) *Reading the fat column.*—Measure with a pair of dividers and read (*a*) from the extreme bottom to the extreme top, (*b*) from the extreme bottom to the lower line of the meniscus. Record and report both results. In cases where a 9-gram sample has been taken for the test, add the two readings (*a*) and (*b*) to obtain the percentage of fat.

(5) *Experience.*—In the case of all methods make preliminary trials. Repeat as many times as seem necessary in order to become adequately prepared for the regular determinations to be made on selected samples. The importance of ample experience cannot be overestimated, especially in connection with the Roese-Gottlieb method.

(6) *Plan of work.*—The following outline will serve to indicate the determinations which are to be made on the various samples named in the heading.

MILK	CREAM	EVAPORATED MILK	ICE CREAM
Roese-Gottlieb	Roese-Gottlieb	Roese-Gottlieb	Roese-Gottlieb
Wendler	Wendler	Wendler	Wendler
Babcock	Babcock	Brinsmaid	Grigsby

The results reported by the collaborators have been compiled and arranged for purposes of comparison as shown in the following tables.

The results shown in Tables 1 and 2 indicate chiefly a comparison of the Wendler and Brinsmaid methods with the Roese-Gottlieb as described in the instructions to the collaborators. In methods directing the use of a 9-gram sample of evaporated or condensed milk the collaborators were instructed to compute the fat percentages by three different formulas. A comparison of the results reported by these methods indicates that the sum of the readings designated (*a*) and (*b*) represents a fair basis on which to make the comparisons. The results obtained by multiplying reading (*a*) by 2 and deducting 0.24 were for the most part apparently too low. This method of expressing the fat measurement was, however, originally intended for tests applied to ice cream. With very few exceptions, the results obtained by multiplying reading (*b*) by 2 and adding 0.15 agreed substantially with the results obtained by adding fat-column readings (*a*) and (*b*). It therefore has been decided, for purposes of comparison of results by the various methods, to confine the Wendler and Brinsmaid figures to the sum of readings (*a*) and (*b*). Results by various miscellaneous methods are also shown in the tabulations.

Mr. J. T. Keister, of the dairy laboratory, U. S. Bureau of Chemistry, has made a special study of the methods which were described in the instructions to the collaborators. These methods have been subjected to a large number of comparative tests on samples of milk, cream, evaporated

TABLE 1.

Fat determinations in condensed milk.

COLLABORATORS' SAMPLE No. 1—EVAPORATED MILK.

ANALYST	ROESE-GOTTLIEB	WENDLER	BRINSMAID	OTHER METHODS
H. B. Burnett	8.05	8.10	8.20	
R. E. Stevenson	8.06	7.65	7.85	
C. Bahlman	[1]8.17	8.05	8.15	
C. G. Sutton	7.98			
	7.96	7.95	8.15	
	7.93	7.96	8.15	
	7.94			
G. B. Taylor			7.60	
	[1]7.56		7.85	
H. S. Bailey	8.06			
E. W. Thornton	8.01	8.10	7.90	
C. L. Black	[1]7.63		7.95	
	[1]7.66		8.10	
C. N. Austin	7.81	8.21	8.14	[2]7.58
	7.81	8.12	8.13	[2]7.40
	7.83	8.16	8.12	[2]7.62
	7.82	8.14		[2]7.78
	7.81			[2]7.54
				[2]7.32
W. D. Strack	7.98			
	7.97			[3]8.3
	7.98			[3]8.3
J. H. Bornmann	7.95		8.00	[2]8.05
	7.92		7.95	[2]7.96
F. C. Broeman	[1]7.76	7.85	7.95	
F. F. Fitzgerald	7.98			
	7.97			
	7.96			
	7.96			
	7.96			
H. M. Miller	7.98			
	7.99			
	8.00			
	8.03			
P. J. Donk	7.96			
	7.97			
	7.97			
	7.94			
Indiana Condensed Milk Co.	7.98			
	7.98			
W. C. Geagley	7.97	6.8	8.0	[4]8.0
	8.00	5.8	8.0	[4]8.0
C. L. Munroe	7.97			
	7.94			
	7.86			
	7.90			
H. E. Otting			8.20	
	8.10		8.10	[3]8.60
H. A. Halvorson	7.94			
	7.89			
J. T. Keister	8.05			[5]8.08
	8.06			[5]7.96
O. L. Evenson				[5]8.02
	8.01			[5]8.05
Maximum	8.10			
Minimum	7.81			
Average	7.96			

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[1] Not included in calculations.
[2] Grigsby method.
[3] Beimling method.
[4] Hunziker method.
[5] Biesterfeld-Evenson method.

TABLE 1.—*Continued.*

COLLABORATORS' SAMPLE No. 2—SWEETENED CONDENSED MILK.

ANALYST	ROESE-GOTTLIEB	GRIGSBY	BRINSMAID	WENDLER	OTHER METHODS
L. B. Burnett	9.05	9.05			
R. E. Stevenson	9.13	9.30			
C. Bahlman	9.07		8.35	8.20	
C. G. Sutton	9.10				
	9.13				
G. B. Taylor	8.96				
H. S. Bailey	9.00				
E. W. Thornton	[1]9.38				
C. L. Black	[1]9.49				
	[1]8.57				
C. N. Austin	9.14	8.80	9.20		
	9.10	8.92	9.11		
	9.11	8.81	9.18		
		9.04	9.24		
W. D. Strack	9.09				
	9.08				[2]9.2
	9.07				[2]9.1
J. H. Bornmann	9.17				[3]8.1
	9.12				[3]8.4
E. C. Broeman	[1]7.95				
F. F. Fitzgerald	9.07				
H. M. Miller	9.09				
	9.09				
	9.09				
	9.08				
P. J. Donk	9.07				
	9.08				
C. L. Monroe	[1]9.58				
	[1]8.62				
J. T. Keister	[1]9.24				[4]9.20
	[1]9.245				[4]9.25
O. L. Evenson	9.12				[4]9.12
	9.05				[4]9.12
H. A. Halvorson	9.08				
	8.99				
Maximum	9.17				
Minimum	8.96				
Average	9.08				

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[1] Not included in calculations.

[2] Beimling method.

[3] Leach method.

[4] Biesterfeld-Evenson method.

TABLE 1.—*Continued.*

COLLABORATORS' SAMPLE No. 53N8A—SWEETENED CONDENSED MILK.

ANALYST	ROESE-GOTTLIEB	GRIGSBY	BRINSMAID	OTHER METHODS
L. B. Burnett	[1]8.95	9.20		
R. E. Stevenson	[2]9.15	8.80		
C. G. Sutton	8.86			[3]8.85
	8.84			[3]8.92
G. B. Taylor	8.85			
H. S. Bailey	8.99			
E. W. Thornton	[2]8.69			
C. L. Black	[2]8.06			
	[2]8.04			
C. N. Austin			9.21	
	8.90	8.52	9.12	
	8.90	8.59	9.14	
	8.91	8.81	9.24	
W. D. Strack	8.83			
	8.83			[4]9.10
	8.84			[4]9.00
J. H. Bornmann	8.89			[5]8.55
	8.86			[5]8.40
F. C. Broeman	[2]7.38			
F. F. Fitzgerald	8.97			
	8.95			
H. M. Miller	8.95			
	8.96			
	8.93			
	8.97			
P. J. Donk	8.93			
	8.93			
C. L. Munroe	[2]8.58			
	[2]8.62			
J. T. Keister	[2]9.105			[3]9.116
	[2]9.13			[3]9.075
O. L. Evenson	8.95			[3]9.00
	8.98			[3]9.05
H. A. Halvorson	8.93			
	8.96			
W. C. Geagley	8.81		6.8	[6]6.2
	8.82		6.8	[6]5.9
	8.83			[7]7.2
				[7]7.2
Maximum	8.99			
Minimum	8.81			
Average	8.90			

[1] Average of the following determinations: 8.93, 8.97, 8.94, 8.96, 8.96, 8.94.
[2] Not included in calculations.
[3] Biesterfeld-Evenson method.
[4] Beimling method.
[5] Leach method.
[6] Wendler method.
[7] Hunziker method.

TABLE 2.

Collaborative fat determinations in milk, cream, and ice cream.

MISCELLANEOUS MILKS.

ANALYST	ROESE-GOTTLIEB	WENDLER	BABCOCK	VARIATIONS FROM ROESE-GOTTLIEB	
		Average of (*a*) and (*b*) readings	Average of (*a*) and (*b*) readings	Wendler	Babcock
H. B. Burnett	3.70	3.69	3.52	−0.01	−0.18
	3.79	3.75	3.52	−0.04	−0.27
R. E. Stevenson	3.70	3.60	3.40	−0.10	−0.30
	3.62	3.65	3.45	+0.03	−0.17
C. Bahlman	3.56	3.68	3.60	+0.12	+0.04
	4.69	4.78	4.80	+0.09	+0.11
C. G. Sutton	[1]3.96	[1]4.09	[1]4.00	+0.13	+0.04
G. B. Taylor	3.78	3.65	[1]3.80	−0.13	+0.02
E. W. Thornton	4.58	4.58	4.53	0.00	−0.05
C. L. Black	[1]3.18	[1]3.05	[1]3.23	−0.13	+0.05
	[1]4.18	[1]4.04	[1]4.28	−0.14	+0.10
J. H. Bornmann	[1]3.32	[1]3.33	3.25	+0.01	−0.08
	[1]3.56	[1]3.28	3.55	−0.28	−0.01
H. A. Halvorson	4.61	4.65	4.55	+0.04	−0.06
	3.06	3.05	3.00	−0.01	−0.06
W. C. Geagley	3.45	3.40	3.43	−0.05	−0.02
MISCELLANEOUS CREAMS.					
H. B. Burnett	18.25	18.00	17.60	−0.25	−0.65
	18.37	18.80	17.60	+0.43	−1.20
R. E. Stevenson	18.05	18.40	17.48	+0.35	−0.57
	18.45	19.20	18.40	+0.75	−0.05
C. Bahlman	17.69	17.88	18.50	+0.19	+0.81
	11.13	11.25	11.50	+0.12	+0.37
C. G. Sutton	[1]22.15	[1]22.90	[1]23.00	+0.75	+0.85
G. B. Taylor	16.88	15.25	[1]18.50	−1.63	+1.62
E. W. Thornton	29.21	31.00	31.00	+0.79	+0.79
C. L. Black	[1]16.80	[1]16.35	[1]16.88	−0.45	+0.08
	[1]15.41	[1]15.08	[1]15.43	−0.33	+0.02
J. H. Bornmann	[1]16.44	....	[1]18.00	....	+1.56
	[1]19.35	....	[1]19.80	....	+0.45
W. C. Geagley	16.37	17.10	17.00	+0.73	+0.63

MISCELLANEOUS ICE CREAMS.

ANALYST	ROESE-GOTTLIEB	WENDLER	GRIGSBY	VARIATIONS FROM ROESE-GOTTLIEB	
		Sum of (*a*) and (*b*) readings	Sum of (*a*) and (*b*) readings	Wendler	Grigsby
H. B. Burnett	9.11	9.40	9.20	+0.29	+0.09
	6.04	5.60	5.80	−0.44	−0.24
R. E. Stevenson	9.00	8.84	9.15	−0.16	+0.15
	6.30	5.23	5.13	−1.07	−1.17
C. Bahlman	11.02	10.60	10.75	−0.42	−0.27
C. G. Sutton	[1]11.63	[1]11.45	[1]11.75	−0.18	+0.12
G. B. Taylor	[1]13.47	13.00	[1]13.80	−0.47	+0.33
E. W. Thornton	14.77	14.80	14.66	+0.03	−0.11
C. L. Black	[1]11.06	[1]10.13	[1]10.90	−0.93	−0.16
	[1]11.03	[1]9.83	[1]10.93	−1.20	−0.10
J. H. Bornmann	[1]9.63	....	[1]9.73	....	+0.10
	10.29	....	[1]9.98	....	−0.31
H. A. Halvorson	[1]10.46	10.70	10.30	+0.24	−0.16
	[1]12.28	12.40	12.60	+0.12	+0.32
W. C. Geagley	[1]11.31	[1]11.40	[1]11.20	+0.09	−0.11

[1] Average of two or more results.

milk, and ice cream. The report of Mr. Keister as a collaborator during the past season shows a large amount of painstaking thorough work, and is so extensive and complete as to necessitate its incorporation herewith separately under Tables 3 and 4.

The following comments have been submitted by the collaborators:

COMMENTS BY COLLABORATORS.

C. L. Munroe, Miner Laboratories, Chicago, Ill.: Was unable to obtain any results by the Wendler method. In only one or two instances (while working with other samples of unsweetened condensed milk) was I able to obtain a fat column and those obtained were very unsatisfactory, as they were not clear and did not have a definite lower limit. In all other cases no fat column at all was obtained. I then tried several minor variations in the procedure outlined in the written directions, as follows: After mixing the sample, water, reagent, and isobutyl alcohol (this mixing was done very carefully and thoroughly in every case, shaking each bottle five minutes), I heated the bottles in a water bath at 55°C. for 20 minutes, with shaking. Then centrifuged at high speed for 5 minutes, added boiling water up to the neck, centrifuged 2 minutes at high speed, added boiling water to bring fat column up into neck, centrifuged 1 minute slowly and 1 minute at high speed. Obtained a fat column, but it was not clear, contained lumps of casein, and the lower limit was not sharply defined.

W. D. Bigelow, National Canners' Association, Washington, D. C.: When the samples were examined in this laboratory we had mislaid your letter, and therefore we examined them by the method that we ordinarily employ; that is, making only two extractions. It came to my attention afterwards that your letter directed three extractions. The results of Messrs. Fitzgerald, Miller, and Donk are, therefore, 0.02 or 0.03 lower than they would have been if your directions had been followed exactly.

F. C. Broeman, F. C. Broeman & Co., Cincinnati, O.: The Babcock method and its modifications gave such poor results on the sugared evaporated milk that I would not deem them worthy of consideration. Charring and indistinct separations made it impossible to get check readings on different determinations of same sample.

J. H. Bornmann, U. S. Food and Drug Inspection Laboratory, Chicago, Ill.: Brinsmaid method: While this method gives good results, it is objectionable because it requires the coating of the bottle with glycerol and heating in boiling water for 20 minutes.

Grigsby method: This method has the advantage of rapidity and simplicity over the Brinsmaid method. One disadvantage is the difficulty of knowing when just enough, and not too much, acid has been added.

Wendler method: Whereas this method gave satisfactory results on milk, it was a failure on all the other products. The fact that a comparatively harmless solution at moderately high temperature is used in place of sulphuric acid at a high temperature is one point in favor of this method. The fat column in the case of milk was very clear and sharply defined, which was not often the case to so marked a degree with the other methods employed.

The Roese-Gottlieb method requires a longer lapse of time before the results are obtained, but the total time spent on the manipulation is not so great when compared with the time spent on a centrifugal method that gives trouble. It is evident that the fat can be weighed more accurately than measured.

The Leach method (precipitation with copper sulphate and removal of the sugar solution in sweetened milk) seems to give low results.

H. A. Halvorson, State Dairy and Food Department, St. Paul, Minn.: After a large amount of preliminary work I found that I was able to obtain very satisfactory results with the Wendler method on whole milk, evaporated milk, and ice cream; with the Brinsmaid method on evaporated milk; and with the Grigsby method on ice cream. The importance of sufficient preliminary experience with all these methods, especially the Wendler method, cannot be overestimated. The following paragraphs show briefly the results that can be obtained. All the results are averages of closely agreeing duplicates.

Wendler method: On three samples of miscellaneous whole milks, results (the average of (*a*) and (*b*) readings) on two samples ran 0.04 and 0.09% higher and results on one sample ran 0.01% lower than the corresponding results by the Roese-Gottlieb method. On four samples of miscellaneous evaporated milks, results (sum of (*a*) and (*b*) readings) on three samples ran 0.02, 0.03, and 0.01% higher and results on one sample ran 0.04% lower than the corresponding Roese-Gottlieb results. On six samples of miscellaneous ice creams, results (sum of (*a*) and (*b*) readings) on five samples ran 0.21, 0.12, 0.30, 0.04, and 0.24% higher and results on one sample ran 0.14% lower than the corresponding Roese-Gottlieb results.

Brinsmaid method: On four samples of miscellaneous evaporated milk, results (sum of (*a*) and (*b*) readings) ran 0.22 and 0.08% higher and results on the other two samples ran 0.09 and 0.09% lower than the corresponding Roese-Gottlieb results.

Grigsby method: On five samples of miscellaneous ice creams, results (sum of (*a*) and (*b*) readings) on four samples were 0.21, 0.32, 0.35, 0.26% higher and results on one sample were 0.01% lower than the corresponding results by the Roese-Gottlieb method.

Roese-Gottlieb method: I consider this method superior to any other when great accuracy is required. An experienced analyst, with care, can obtain results that agree very closely, say, within less than 0.1%. In order that different analysts may obtain concordant results on the same sample, it is more desirable (due to errors made in measuring) to weigh the sample directly rather than to take 10 cc. of solution containing 40 grams in 100 cc. This is especially true in the case of ice cream, which usually contains a large amount of air even after being melted. I think that results nearer the truth would be obtained if the directions required that the sample be weighted out directly.

E. W. Thornton, Department of Agriculture, Raleigh, N. C.: I experienced some difficulty in the Wendler method on ice creams, due apparently to slight saponification of the butterfat. The results reported on the ice cream by this method are on a pure ice cream which contained no thickening agent.

My results on evaporated milk are on the sample No. 1 (unsweetened) which was sent to me. Both centrifugal methods on this product gave very clear and satisfactory fat readings, but with a uniform difference of 0.2% between readings (*a*) and (*b*). I was unable to obtain satisfactory reults by either the Wendler or Brinsmaid methods on sweetened condensed milk.

C. N. Austin, Sears, Roebuck & Co., Chicago, Ill.: Roese-Gottlieb method: In this work the fat flasks were dried to constant weight before the ether extracts were added. A counterpoise flask was used, which underwent the same heating as the flasks used in weighing the fat. It was found to be impossible to weigh a series of flasks with uniform rapidity. To compensate for the change in weight of the flasks, which takes place when they are removed from the desiccator, each one was allowed to stand on the balance pan until constant weight had been reached before a weighing was recorded. Another error that was difficult to avoid was caused by casein or water-soluble material passing through the filter with the ether extracts. To avoid this the extracts were allowed to stand about 30 minutes, and the filters were moistened with petroleum ether before adding the extracts. In making the extractions petroleum ether redistilled and coming over below 60° was used. Blanks were run to allow for the extractive matter in the reagents and nonvolatile residue in the ether. These caused a correction of 0.02 to 0.04 in the percentage. We were able to obtain results checking within 0.05%.

Babcock method: The modifications of the Babcock methods suggested were used in making determinations of the fat on the three samples submitted, as well as on a number of samples used for preliminary work. Bottles of the form specified could not be obtained in time for the work. The bottles used were of the ordinary form, reading to 10% and graduated to fifths of a per cent. The height from 0 to 8.0 on the stem of the 10% bottles was 1.5 cm. less than the height of the corresponding reading of the 8% bottles. A number of determinations were afterward made on other samples using the two kinds of bottles. This showed an a erage difference in the height of meniscus for the two kinds of bottles of only 0.01 on the scale. No correction was applied, however, since it was ascertained that the correction of −0.24, recommended for the Brinsmaid method, was originally determined from results obtained with bottles of the kind used by us. With all of the Babcock methods it was found difficult to obtain a sharply defined column of clear fat, due sometimes to a slight char, but more often to the presence of a slight amount of fine caseous material that prevented an accurate reading of the lower limit of the fat column. The fact that errors are multiplied by the necessity of using 9-gram samples would appear to be an added objection to the Babcock methods.

The Brinsmaid method was found to give results higher than the Roese-Gottlieb results, even after the correction had been applied. However, the difference was not uniform for the three samples. The method gave results that check closely within themselves, and seemed to be as good for sweetened condensed milk as for evaporated milk.

The Grigsby method was found to be very unsatisfactory. It was very difficult to obtain fat columns free from curd or char, and even perfectly clear fat columns would sometimes give readings varying by as much as 0.2. The results were lower than those obtained by the other Babcock methods. With sweetened condensed milk this method was only applicable when the reagents were added to the drained curd. The presence of a small amount of water prevented the complete solution of the caseous material. When the results were tabulated the correction for the Brinsmaid method was applied to these results also as indicated in the instructions.

In the case of the Wendler method we substituted amyl alcohol for the isobutyl alcohol recommended, since the latter could not be obtained, and the former appeared to be effectual in preventing saponification of the fat. The method thus modified gave results with evaporated milk that accorded closely with those obtained by the Brinsmaid method, but with sweetened condensed milk no results could be obtained, since the precipitated curd did not dissolve completely.

H. S. Bailey, Bureau of Chemistry, Washington, D. C.: Just for my own satisfaction I tried to see how close duplicates could be obtained on a sweetened condensed milk, weighing up the original milk after dilution instead of merely using a 10 cc. pipette. Somewhat to my surprise the results were 8.996, 8.994, and 8.992. By using the weighing burette so that only one weighing more than the number of portions weighed out is required, very little more time is required than if we used a pipette, as there is no occasion for rinsing. To my mind the one drawback in the Roese-Gottlieb method as outlined is the danger of using too much water in rinsing out the pipette. I find that if only 2 or 3 cc. are used there is no emulsion formed, while if one takes 10 cc. it is necessary to let the determination stand for a long time before it is possible to draw off the ether-fat column.

L. B. Burnett, Bureau of Chemistry, Washington, D. C.: Of the two centrifuge methods tried on whole milk, the Wendler method gave the best results, the fat column being perfectly clear, the lower meniscus horizontal. On evaporated milk the Wendler method did not work quite as well as on whole milk. More consistent results were obtained if the sample was not shaken during the heating, but rotated. With a little experience good results were obtained. On cream and ice cream the Wendler method seemed to give satisfactory results. The variations in the results on the second sample of cream and ice cream were not due to any fault of the method, as clear fat columns were obtained, but to the condition of the sample.

The Grigsby method was found to be satisfactory for sweetened condensed milk. The sample being diluted with one-third its weight of water and 12 instead of 9 grams being weighed out.

The Brinsmaid method did not appear to offer any special advantages, but satisfactory results were obtained with it on evaporated milk.

In the Wendler method in making up the fat column to the top of the graduations, it was found that if a dilute alcohol was employed instead of water the flocculent material was less apt to come to the top; however, this is not necessary if the sample is not shaken violently during the heating.

C. L. Black, U. S. Food and Drug Inspection Laboratory, Philadelphia, Pa.: I found the Wendler method most unsatisfactory in that in no case was I able to get all the curd dissolved by the amount of heating and shaking directed in the method, this being especially noticeable in the case of ice cream. The method would appear very unsatisfactory as a routine laboratory method in that, owing to the amount of vigorous shaking required, so few determinations can be made at one time, and these require so much more attention from the analyst than any of the ordinary methods used for similar products. In addition, it appears that the prolonged shaking seems to cause a decided loss of fat.

Geo. B. Taylor, State Board of Health, New Orleans, La.: Wendler method proved to be very unsatisfactory. In nearly every instance the clear fat would be underlain by curd. In a few instances the fat refused to rise.

It is my opinion that the Roese-Gottlieb method as outlined (40 grams to 100 cc., 10 cc. = 4 grams taken) is unsatisfactory where materials heavy in fat are concerned. Small lumps of fat will invariably form when the solution is mixed, and remain in the pipette after the required amount is run into the Röhrig tube. When large amounts of fat are present it is practically impossible to get to constant weight by heating at the temperature of boiling water. An appreciable loss is almost always noted on heating for intervals of an hour.

During the last two years this laboratory has used, with good results, a slight modification of the Grigsby method for analyzing ice cream. We use 17.6 cc. of glacial acetic acid, mix well, but do not heat, adding commercial sulphuric acid to dark coffee brown color (about 10 cc.). This gives a clear reading. The results duplicate well.

On the other hand, it has been difficult to obtain good duplicates with the Roese-Gottlieb method, especially with fruit ice creams. No method is satisfactory which requires the weighing out of 9 grams of sweetened condensed milk into an 8% Babcock milk bottle. This was done on triplicate samples, and worked by the Brinsmaid method, with no fat rising. A 40% solution was made up and 15 cc. equivalent to 6 grams were taken. No results were obtained. The Roese-Gottlieb method is the only satisfactory method for sweetened condensed milk.

Clarence Bahlman, Board of Health, Cincinnati, Ohio.—When 18 grams of cream were treated with Wendler's reagent, there was a slight curd under the fat, when heated 5 minutes at 50°C. This was entirely avoided when the mixture of cream and reagent was heated 10 minutes at 60°C. In the case of the evaporated milk, the same trouble was experienced when heating 5 minutes at 50°C., but was remedied by heating 10 minutes at 60°C. With the sweetened condensed milk, the Brinsmaid method gave considerable trouble. The fat layer showed a dark curd. Four tests were made; in only one were we able to obtain a satisfactory fat. Also, the Wendler method showed some curd when heating 5 minutes at 50°C., but gave clear separation when heated 10 minutes at 60°C.

J. T. Keister, Bureau of Chemistry, Washington, D. C.: In the Brinsmaid method very good readings were obtained in most cases and the results indicate that it gives figures varying only about 0.10 to 0.15 from the truth, when the readings are made to lower line of meniscus.

The Wendler method was a failure for evaporated milk on the 9-gram charge. Using a 6-gram charge was a considerable improvement, but as only two samples were tested by this modification no conclusion can be drawn.

The Grigsby method gave very satisfactory results on plain and nut ice cream and is about all that could be expected of a centrifugal method. In case of plain cream not a sufficient number of figures have been recorded to form any conclusion as to whether the correction −0.24 is necessary or not, as only two samples were examined, with some variation in results. In case of nut ice creams these results indicate clearly that the above correction is not necessary. The method worked successfully on the sample of pineapple cream, but in case of the other fruit creams (peach and strawberry) the method was not a success, the fat not being completely liberated and the fruit pulp also interfering with readings.

In case of the Wendler method, the results show that in its present form the method is not applicable to ice cream.

DISCUSSION OF RESULTS.

Reports from collaborators, most of whom reported determinations in duplicate, are on the whole a very satisfactory demonstration of the reliability of the Roese-Gottlieb method, especially when applied to such products as unsweetened and sweetened evaporated milk. With the exception of a few modifications, as suggested by some of the collaborators, the directions for carrying out the Roese-Gottlieb method will doubtless remain in the form already adopted as provisional. The preferred modification consists in weighing the sample. With this modification and one or two not very material changes in description, the method is in form for another season's study, anticipating its final adoption as official.

The associate referee selected, finally, for comparative study the methods devised by Wendler,[1] Brinsmaid,[2] and Grigsby.[2] The first of these, it is true, was described as applicable to the testing of milk, but preliminary trials of the method on other products led to the hope that good results might be forthcoming if the collaborators were directed to apply it to all samples submitted. The Brinsmaid method was devised for evaporated milk and the Grigsby method for ice cream. Unfortunately, in planning the work for the present year, the method described by Hunziker, and recommended for study at the meeting held in 1909, was overlooked.

[1] Z. öffent. Chem., 1906, **12**: 41–58; Analyst, **31**: 118.

[2] Correspondence from Mr. David Klein of the Illinois Food Commission.

There are, however, some results by this method reported by two of the collaborators, and these, together with information received from reliable sources, afford a basis for the belief that the method should be included in the coöperative study to be carried out next year. So far as can be judged from results reported by the collaborators, it appears that the methods devised by Brinsmaid, Grigsby, and Hunziker are worthy of further study, attention being given primarily to the products for which they were designed; and, in addition to these, there may probably be included the methods described by Beimling and Manchester. In the selection of methods it will, however, be borne in mind that a test like that described by Beimling has the serious defect arising from the probable solution of amyl alcohol in the fat column, thus causing too high results. A modified Roese-Gottlieb procedure has for some time been under process of perfection in the dairy laboratory at the U. S. Bureau of Chemistry, but there is no assurance as yet that this is available for collaborative study with a view to adoption by this association. It may seem like a venture somewhat far from the aims of the association to put these modified centrifugal methods so much to the fore in our collaborative work in comparison with a method now well-nigh permanently established. There are, however, two purposes in view, either one of which seems to justify the past season's plan of work. In the first place, there is a persistent demand for a reliable quick method for factory control work or as a reliable sorting-out test for use in State and municipal food and dairy laboratories. In the second place, in the interest of sound policy and future stability in our work as analysts, this group of miscellaneous proposed so-called modified Babcock tests should be subjected to careful criticism and fair treatment. Then, any of these modifications that are not to be depended upon for the purposes for which they were devised should be consigned to oblivion. The supply of such methods in the last half dozen years has perhaps much exceeded the demand; most of them have gone into print without anything like adequate analytical backing; and the originators have been for the most part overconfident and somewhat lax in their descriptions. Some analysts, in fact, seem to imagine that one or two of these methods are applicable to all kinds of dairy products, from plain milk to ice cream and infant foods. The program which will be offered for the coming year will at any rate contemplate a weeding out among the score or more of these centrifugal methods with a view to determining which, if any, are really dependable for the products for which they were originally designed.

RECOMMENDATIONS.

It is recommended—

(1) That the Roese-Gottlieb method be adopted as official for the determination of fat in milk and condensed milk, both unsweetened and sweetened.

(2) That a further study be made of the Roese-Gottlieb method in the analysis of ice cream, milk powders, malted milks, and milk chocolates.

(3) That a special further study be made of modifications of the Babcock method as applied to condensed milk, both unsweetened and sweetened, and to ice cream.

TABLE 3.

Comparative study of fat methods for milk, cream, and evaporated milk.

MILK.

SAMPLE	WENDLER METHOD[1]				BABCOCK METHOD				ROESE-GOTTLIEB METHOD
	(a)		(b)		(a)		(b)		
	Reading	Per cent fat	Reading	Per cent fat	Reading	Per cent fat	Reading	Per cent fat	Per cent fat
No. 1........	3.95	3.95	3.75	3.75	3.75	3.75	3.55	3.55	3.69 3.696
No. 2........	3.40	3.40	3.20	3.20	3.20	3.20	3.00	3.00	3.225 3.218

[1] Reading O. K.

CREAM.

METHOD	READING	PER CENT FAT	READING	PER CENT FAT	READING	PER CENT FAT
	(a)		(b)		(c) with glymol	
Wendler:[1]						
No. 1............................	17.9	17.88	17.30	17.29	17.40	17.39
No. 2............................	16.4	16.12	15.8	15.58	15.7	15.48
Babcock:						
No. 1............................	17.9	17.74	17.3	17.15	17.4	17.25
No. 2............................	15.9	15.70	15.3	15.11	15.4	15.20

[1] Found it difficult to get uniform readings with cream. Method not considered as reliable as Babcock.

Roese-Gottlieb:	Per cent fat
No. 1..	17.32
No. 2..	15.16 15.23

TABLE 3.—*Continued.*

EVAPORATED MILK.

SAMPLE	WEIGHT OF SAMPLE	READINGS		PER CENT FAT				REMARKS
		(a)	(b)	[1](a)×2	(b)×2	(a)×2 −0.24	(b)×2 +0.15	
				BRINSMAID METHOD.				
	grams							
a	9.0947	3.8	3.6	7.52	7.12	7.28	7.27	Readings O. K.
	9.0720	3.8	3.6	7.54	7.14	7.30	7.29	Slight undissolved curd.
b	9.0622	3.8	3.6	7.54	7.15	7.30	7.30	Some charring.
	9.0600	3.9	3.7	7.74	7.35	7.50	7.50	Do.
c	9.0747	3.9	3.7	7.73	7.33	7.49	7.48	Reading O. K.
	9.0550	3.9	3.7	7.75	7.35	7.51	7.50	Little brown "mud."
d	9.0395	3.9	3.7	7.76	7.36	7.52	7.51	Cloudy fat but good reading.
	9.9780	3.9	3.7	7.73	7.33	7.49	7.48	Do.
e	9.1914	4.4	4.2	8.61	8.22	8.37	7.91	Very good readings.
	8.9388	4.3	4.1	8.66	8.25	8.42	8.02	Do.
f	9.0417	4.10	3.90	8.16	7.76	7.92	7.91	Readings O. K.
	9.0276	4.15	3.95	8.26	7.87	8.02	8.02	This one charred.
g	9.0305	4.40	4.20	8.77	8.37	8.53	8.52	Slight char; not perfect.
	9.1270	4.45	4.25	8.776	8.38	8.54	8.53	Do.
h	9.0245	4.3	4.1	8.57	8.17	8.33	8.32	Some char.
	9.1210	4.2	4.0	8.29	7.89	8.05	8.04	Reading O. K.
				WENDLER METHOD.				
a	9.0437	3.45	3.25	6.86	6.46	6.62	6.61	Undissolved casein, not a success.
	9.0722	3.30	3.10	6.54	6.15	6.30	6.30	
b	9.1882	3.55	3.35	6.95	6.56	6.71	6.71	Some white curd.
	9.0677	3.50	3.30	6.94	6.55	6.70	6.70	Do.
c	9.1330	3.20	3.00	6.37	5.98	6.13	6.13	Much white curd.
	9.0575	3.10	2.90	6.18	5.77	5.94	5.92	Do.
i	6.0700	2.60	2.40	7.71	7.12	7.47	7.27	Clear reading; (a) × 3.
	6.2470	2.70	2.50	7.77	7.20	7.53	7.35	Do.
j	6.0235	2.50	2.30	7.48	6.87	7.24	7.04	Reading O. K.; (a) × 3.
	6.0651	2.50	2.30	7.42	6.82	7.18	6.97	Slight brown "mud."

ROESE-GOTTLIEB METHOD.

SAMPLE	PER CENT	SAMPLE	PER CENT	SAMPLE	PER CENT	SAMPLE	PER CENT
a....	7.172	c....	7.41	e....	8.172	h....	8.05
	7.144		7.41		8.197		8.06
b....	7.10	d....	7.478	f....	7.765	i......	7.12
	7.15		7.519		7.779	j......	7.457
				g......	8.59		

[1] (a) is corrected to 9 grams.

TABLE 4.

Comparative study of fat methods with ice cream.

GRIGSBY METHOD.

SAMPLE	VARIETY	WEIGHT OF SAMPLE. (GRAMS)	READINGS		PER CENT FAT					REMARKS
			(a)	(b)	(a) + (b)[1]	(a) × 2	(b) × 2	(a) × 2 − 0.24	(b) × 2 + 0.15	
a	Vanilla.....	9.0915	4.95	4.78	9.62	9.80	9.40	9.56	9.55	Reading O. K.
		9.2712	5.10	4.90	9.70	9.90	9.51	9.66	9.66	Do.
b	Chocolate	9.0716	4.55	4.35	8.82	9.028	9.60	8.78	8.75	Reading perfect.
		9.1961	4.60	4.40	8.82	9.04	8.62	8.80	8.77	Few small lumps of chocolate in neck; not objectionable.
c	Vanilla.....	9.0130	4.60	4.40	8.97	9.18	8.78	8.94	8.93	Reading O. K.
		9.0232	4.70	4.50	9.16	9.37	8.97	9.13	9.12	Do.
d	Pineapple..	9.0484	4.05	3.85	7.86	8.06	7.66	7.82	7.81	Fruit pulp was in neck of bottle, but separated from fat, and did not interfere with readings.
		9.0067	4.10	3.90	7.98	8.19	7.77	7.95	7.93	Do.
e	Peach......	9.2447	3.50	3.30	6.61	6.81	6.42	6.57	6.57	Fat and pulp not in contact; no interference with reading.
		9.1150	3.50	3.30	6.71	6.91	6.50	6.67	6.65	Fairly good reading; pulp interfered somewhat.
f	Strawberry.	9.0820	4.00	3.80	7.73	7.93	7.52	7.69	7.67	Only fair reading, pulp interfered.
		9.1520	4.20	4.00	8.06	8.26	7.86	8.02	8.01	Do.
g	Maple nut..	9.5625	4.70	4.50	8.65	8.84	8.46	8.60	8.61	Reading O. K.; nuts in neck of bottle, but separated from fat.
		9.0225	4.40	4.20	8.58	8.78	8.38	8.54	8.53	Very good reading obtained by heating H_2O bath slightly above 60°, which caused separation of nuts from fat.
h	Peach......	9.0210	3.90	3.70	7.58	7.78	7.38	7.54	7.53	Fruit pulp interfered with readings, some fat being entangled with pulp.
		9.2230	3.8	3.60	7.27	7.52	7.02	7.78	7.17	Do.

WENDLER METHOD.

SAMPLE	VARIETY	WEIGHT OF SAMPLE. (GRAMS)	(a)	(b)	(a) + (b)[1]	(a) × 2	(b) × 2	(a) × 2 − 0.24	(b) × 2 + 0.15	REMARKS
a	Vanilla.....	9.1160	4.30	4.10	8.28	8.49	8.08	8.25	8.23	Only fair test some curd.
		9.0710	4.5	4.30	8.72	8.92	8.52	8.68	8.67	Too much curd to read accurately.
b	Chocolate	9.0855	4.45	4.25	8.61	8.81	8.42	8.57	8.57	White "mud" at bottom of fat column; not a success.
		9.1282	4.50	4.30	8.68	8.87	8.48	8.63	8.63	Do
c	Vanilla.....	9.1370	4.60	4.40	8.86	9.06	8.66	8.82	8.81	White "mud" reading only approximate.
		9.0612	4.50	4.30	8.74	8.94	8.54	8.70	8.69	Do.
d	Pineapple..	9.1090	4.00	3.80	7.70	7.90	......	7.66	......	White curd or "mud" at bottom of fat column.
		9.0730	4.00	3.80	7.74	7.93	......	7.69	......	Do.
e	Peach......	9.0890	3.25	3.05	......	6.44	......	......	......	White curd or "mud;" not a success.
		9.1540	3.25	3.05	......	6.45	......	......	......	Do.

ROESE-GOTTLIEB METHOD.

SAMPLE	VARIETY	PER CENT FAT	SAMPLE	VARIETY	PER CENT FAT
a	Vanilla.......................	9.58 9.57	e	Peach.........................	7.63 7.66
b	Chocolate.....................	9.02 9.03	f	Strawberry....................	8.136 8.138
c	Vanilla.......................	9.216 9.28	g	Maple nut.....................	8.75 8.776
d	Pineapple.....................	8.14 8.16	h	Peach.........................	8.47 8.508

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[1] Corrected to 18 grams.

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